ACTA PHYSIOLOGICA SCANDINAVICA

VOL. 38

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VOL. 38 FASC. 1

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From the Laboratory for the Theory of Gymnastics and the Zoophysiological Laboratory, University of Copenhagen.

Physiological Dead Space and Alveolar Gas Pressures at Rest and during Muscular Exercise.

By

ERLING ASMUSSEN and MARIUS NIELSEN.

Received 8 July 1956.

The knowledge of the correct values of the respiratory dead space to be used in the BOHR formula for the calculation of the average composition of the alveolar air is of great importance, but much disagreement has existed, and still seems to exist, among physiologists as to the magnitude of the change which occurs in the volume of the dead space at the transition from rest to muscular work.

Originally the dead space was thought to be a constant value, but in 1912 DOUGLAS and HALDANE found that the dead space during muscular work undergoes large changes. From a value of 160 cc during rest it was found to increase regularly to well above 600 cc at a work intensity involving a ventilation of 61 liters per minute and a depth of respiration of 3.2 liters. Large increases in dead space was found also during CO_2 breathing at rest (CAMPBELL, DOUGLAS and HOBSON 1914) and during deep breathing at rest produced voluntarily by slowing the frequency of breathing (HALDANE 1915 and HENDERSON, CHILLINGWORTH and WHITNEY 1915). The dead space was in these investigations calculated from the composition of the alveolar air, which was determined by the direct sampling method of HALDANE and PRIESTLEY (1905). When calculated from the oxygen values the dead space was found to be still larger than when calculated

from the CO_2 values. HALDANE and PRIESTLEY's results were challenged by KROGH and LINDHARD (1913 and 1917), who determined the dead space by means of a modification of the hydrogen method of SIEBECK (1911), which was varied and tested in several ways. It was found that the dead space at varying pulmonary inflations below the resting position of the chest remained constant and was of about the same size as that observed by HALDANE and associates for normal breathing at rest. At larger pulmonary inflations, however, the dead space showed a comparatively small increase, which was assumed to be due to a passive stretching of the air passages. At maximum inflations of the lungs the increase amounted to about 100 cc. Muscular work did not produce any increase, except in so far as the airways were passively stretched by the deep inspirations.

It had been pointed out by KROGH and KROGH (1910) and by KROGH (1913), that the average composition of the alveolar portion of the expired air, which is the one that is calculated by the BOHR formula (with a correct volume for the dead space), and the average composition of the air in the alveoli are not the same. The CO_2 pressure of the alveolar air, which shows the lowest value at the end of inspiration but the highest value only some time after the beginning of inspiration when the alveolar air standing in the air passages has been reinspired, will always have a higher average value than the CO_2 pressure of the alveolar portion of the expired air. KROGH proposed that the latter simply be termed "alveolar expired air". KROGH and LINDHARD (1914) investigated the variations in the composition of the alveolar air occurring during the respiratory cycle both at rest and during muscular work by means of fractional analysis of single breaths, and found that the differences between the percentages of both CO_2 and O_2 in the average alveolar air and in the alveolar expired air were small and practically constant. It was therefore concluded, that the best way to determine the composition of the alveolar air in almost all cases is to calculate it by means of BOHR's formula, when the dead space of the subject has been accurately determined.

Further it was concluded (KROGH and LINDHARD 1917) that when the breathing is deep as in muscular work very slight errors only will usually result from not taking the increase in dead space into account, because the increase is small compared to the depth of respiration. KROGH and LINDHARD (1913, 1914

and 1917) pointed out, that the alveolar CO_2 percentage during muscular work as determined by the HALDANE-PRIESTLEY method, owing to the delay which occurs during the sampling and to the increased production of CO_2, is too high, and that the dead space when calculated on the basis of alveolar air obtained by this method consequently also will be too high. HALDANE and PRIESTLEY, on the other hand, maintained that the respiratory dead space when measured by means of an inert gas is not applicable to the problem of the alveolar CO_2 and O_2 pressures, but that the "physiological dead space" must be determined by means of the physiologically active gases.

BANNISTER, CUNNINGHAM and DOUGLAS (1954) have re-investigated the problem of the dead space and the composition of the alveolar air at rest and during muscular exercise. In the experiments with normal breathing at rest the alveolar air was determined by means of HALDANE-PRIESTLEY samples and during CO_2 hyperpnea and during muscular exercise by the end-normal expiration method (LINDHARD 1911 and KROGH 1913) as modified by RAHN and OTIS (1949). It was observed that this method in both conditions gave the same results as the HALDANE-PRIESTLEY method. In CO_2 hyperpnea the dead space was found to be increased up to threefold at a tidal volume of 1.6 liters, and a close agreement was observed between their results and those of CAMPBELL, DOUGLAS and HOBSON (1914) and of COOPER, EMMEL, KOUGH and LAMBERTSEN (1953). In the last mentioned investigation the dead space was calculated on the basis of the arterial pCO_2 instead of the alveolar pCO_2. During the hyperpnea of muscular exercise the dead space rose to about 600 cc. A single point, based on the arterial pCO_2 (RILEY, SHEPARD, COHN and ARMSTRONG 1954 personal communication to BANNISTER, CUNNINGHAM and DOUGLAS) and showing a fairly good agreement with the values based on alveolar pCO_2 was also presented in their graph. From these experiments it was concluded that "The method of sampling which probably reflects most closely the arterial blood gas tensions at rest and the arterial pCO_2 in exercise is that of HALDANE and PRIESTLEY (1905) or some other method based on the same principle. The 'alveolar expired air' of KROGH and LINDHARD (1913 and 1917) is probably misleading, when applied to the problem of respiratory control". It was emphasized however, that the only way to settle the matter finally, was to make direct comparisons between the

arterial blood gas tensions and those of the alveolar air determined by the different methods.

Determination of the dead space in which arterial pCO_2 was used in the BOHR formula instead of alveolar pCO_2 was first attempted by ENGHOFF (1938). In a few experiments with normal breathing and with deep breathing at rest and during muscular work, he found that the ratio of dead space to tidal volume independently of the tidal volume had an average value of 0.34. ROSSIER and BÜHLMANN (1950) also found a constant ratio of about 0.3 between dead space and tidal volume in work experiments with oxygen uptakes up to about 2.4 liters per minute. RILEY et al. (1954) using microtonometrically determined arterial pCO_2 for the calculation measured the dead space in 21 normal subjects of different ages who were studied two or more times during light to heavy exercise (oxygen consumption up to 2.8 liters per minute and tidal volume from about 0.8 liters up to about 3 liters). The results showed the average value of the dead space to be 240 cc with a standard deviation of 111 cc. No correlation, however, was found between the tidal volumes and the widely scattering dead space values. The scatter of results was considered to be due mainly to the errors in estimation of the arterial pCO_2. FILLEY, GREGOIR and WRIGHT (1954) also using microtonometrically determined arterial pCO_2 found like RILEY et al. that the ratio *dead space: tidal volume* decreased considerably during work with increasing respiratory depth.

The studies of COOPER, EMMEL, KOUGH and LAMBERTSEN (1953) on the respiratory dead space during CO_2 breathing at rest were mentioned on page 3.

Also other methods have been used to determine the dead space in rest, during muscular work and during varied depth of breathing. AITKEN and CLARK-KENNEDY (1928) used an apparatus by means of which a single breath during moderate muscular work could be collected and divided into six separate successive portions. The dead space, which was deduced from the CO_2 concentrations in these successive portions by a graphical method, was found to lie between 300 cc and 400 cc. This method was criticized by NIELSEN (1936 p. 111) and by GROSSE-BROCKHOFF and SCHOEDEL (1937). The last mentioned authors with a similar technique but with a different way of calculating the results found that the dead space during muscular work decreased with increasing tidal volumes. Measurements of dead space by means

of continuous analysis of gas content and volume flow of single breaths have been performed by MUNDT, SCHOEDEL and SCHWARZ (1941) using hydrogen, by FOWLER (1948) using nitrogen and by BARTELS, BEER, KOEPCHEN, WENNER and WITT (1954) using oxygen, carbon dioxide, nitrogen and helium as indicator gases. The results obtained by these methods were substantially in agreement with the studies of KROGH and LINDHARD (1917), and BARTELS et al. found furthermore the dead space for all four indicator gases to be the same (with a possible exception in two of the subjects of the helium dead space). PAPPENHEIMER, FISHMAN and BORRERO (1952) introduced a new method based on a graphic solution of the BOHR formula. By this method which required no alveolar gas samples, the dead space and the alveolar expired air could be measured by extrapolation of a curve which related the CO_2 and O_2 pressures of the expired air to the reciprocal of the tidal volumes, the composition of the alveolar air being held constant during the experiments by means of a special procedure. The dead space was found to be the same for CO_2 and for O_2 and to be independent of the variation of tidal volume. It remained also unchanged at a very mild grade of muscular exercise. By using this method FISHMAN (1954) in seven normal subjects found an average value of the dead space of 164 cc. By comparing the gas pressures found in the alveolar expired air with those measured by direct analysis of arterial blood he found an O_2 gradient of 6 mm Hg and a CO_2 gradient of 0.5 mm Hg (range: -6 to $+4$ mm Hg).

In the present investigation determinations of the dead space based on arterial pCO_2 have been made at rest and during muscular work of various intensities both when breathing air and when breathing gas mixtures with low oxygen pressures. Furthermore the gas pressures of "mean alveolar air" have been compared 1) to those of alveolar expired air and, 2) to those of alveolar air as obtained by direct sampling (end-normal expiration method).

Methods and Procedure.

The experiments were performed on a KROGH bicycle ergometer in which the saddle had been exchanged for an armchair, allowing the subject to rest and work in the same semi-reclining position. The pulmonary ventilation and the respiratory metabolism were determined by the DOUGLAS-bag method. The respiratory frequency was registered on a drum. The respiratory valves used for the rest experiments had

been furnished with a diaphragm (VON DÖBELN 1946) by means of which the dead space of the apparatus is reduced to about 10 cc. The valves (plus mouthpiece) used for the work experiments had a dead space of 50 cc. Direct sampling of alveolar air was performed according to the end-normal expiration method of LINDHARD (1911) and KROGH (1913). The samples — taken during the inspiration from a narrow tube just outside the expiratory valve — were collected automatically by the apparatus described by NIELSEN and SMITH (1951). In the work experiments the collection time (time for opening of sampling tube) during each inspiration was shortened by means of a special arrangement. In the low oxygen experiments the subjects inspired from a large DOUGLAS-bag which was continuously filled from a cylinder containing the air mixture (12.3 to 12.7 per cent oxygen in nitrogen). The gas analyses were made on the HALDANE apparatus as modified by KROGH and on the SCHOLANDER apparatus.

The arterial pCO_2 was determined from the pH and the plasma CO_2 content of arterial blood by means of the HENDERSON-HASSELBALCH equation. The samples of arterial blood were collected through a 1 mm cannula inserted into the brachial artery. During collection the blood streamed directly from the artery through a 15 cm long polystyrene tube connected to the cannula down into a sampling tube containing 1 drop of a 5 per cent heparin solution under a layer of oil. The sampling tube was during collection kept in a beaker with crushed ice to which small amounts of water had been added, and the blood, therefore, was cooled immediately to 0° C. After collection the blood and the heparin solution were gently mixed with one another by means of a 10 cc all glass syringe. In order to prevent dilution errors the dead space (about 0.25 cc filled with a diluted heparin solution) of this syringe had previously been flushed with sufficient amounts of the arterial blood. 2.5 or 5.0 cc of the blood was left in the 10 cc syringe for the pH determination, and blood samples for analysis of CO_2 and O_2 content were taken into 1.0 cc syringes. The dead space of these syringes was filled with heparin and the syringes were calibrated to deliver an exactly known volume (about 1 cc) of blood. All syringes were capped and stored at 0° C. The analyses were generally completed within 2 hours of the time of collection.

The carbon dioxide and oxygen content of the arterial blood were determined in duplicate on the VAN SLYKE apparatus. If the CO_2 values differed more than 0.2 volume per cent from one another a third analysis was made. The oxygen capacity of the blood was determined on a sample of arterial blood that had been rotated for 15 minutes in air.

The pH of the blood was determined by means of a *Radiometer* pH meter.[1] The measurements were carried out at ambient temperature in a room with nearly constant temperatures, and the results were

[1] Model PHM3. Glasselectrode (bulb type) number G 160A. The electrode vessel was furnished with a rubber membrane through which the blood could be injected directly from the syringe. — It was found, that for accurate work the use of the millivolt readings is preferable to the use of the direct pH readings.

corrected to the observed body temperature (rectal temperature) by the temperature coefficient of ROSENTHAL (1948). Prior to the measurement the syringe containing the blood sample had been transferred from the water-ice mixture to a water bath which within 0.1° C had the same temperature as the air close to the pH-meter. Measurements showed that even in water it lasted 10 to 15 minutes before a complete temperature equilibrium was reached. The millivolt readings corresponding to the blood sample were compared to those of two phosphate buffers of pH 7,600 and 6,600 at 25° C. The first of these buffers, the pH of which was close to the pH of the arterial blood at room temperature, was measured before and after each measurement on blood.

In order to investigate if with the handling of the blood as here described glycolysis was completely prevented, pH was measured in 16 pairs of blood samples which were all treated as usually, except that sodium fluoride (0.07 per cent in the blood) had been added to one of the samples in each pair. The results showed that pH was slightly higher in the blood samples treated with sodium fluoride, than in the control samples but the average difference was only 0.005. The standard deviation of the differences was $\pm$ 0.003 and the standard error of the mean $\pm$ 0.0008. HAVARD and KERRIDGE (1929) have observed that sodium fluoride is not able to depress glycolysis in heparinized blood. In order to test this possibility 9 pairs of blood samples were placed for two hours at a temperature of 36 to 38° C. The measurements, however, showed an average pH increase of but 0.009 for the blood samples which had been treated both with heparin and sodium fluoride but an average pH decrease of 0.14 for the blood treated only with heparin. The findings of HAVARD and KERRIDGE, for which no explanation was offered, could therefore not be confirmed.

The arterial plasma CO_2 content was estimated from the observed carbon dioxide content, the pH, the oxygen capacity and the oxygen saturation of the blood by means of the line chart of VAN SLYKE and SENDROY (1928) and the arterial pCO_2 was calculated from the pH and the plasma carbon dioxide content by means of the HENDERSON-HASSELBALCH equation. For the pK' and for the solubility coefficient of carbon dioxide in plasma the values of respectively 6.11 and 0.529 at 37° C were used. These values were in each case corrected to the observed body temperature (cp. DILL, DALY and FORBES 1937 and DILL, EDWARDS and CONSOLAZIO 1937).

The validity of the above mentioned indirect way of determining the arterial pCO_2 was tested by analyzing blood with a known pCO_2. This was done by equilibrating different blood samples in tonometers (capacity about 400 cc) with gas mixtures of carbon dioxide in air. The tonometers were rotated in a water bath at 37° to 38° C for 20 minutes, the temperature each time being kept constant with an accuracy of 0.1° C. Heparin and sodium fluoride (0.07 per cent in the blood) were added to the blood. After equilibration the blood was handled in exactly the same way as when determining pCO_2 of arterial blood. The results from the tonometer experiments performed on blood

Table 1.

Tonometer experiments.

Number of experiments	pH	pCO_2			Blood samples
		blood mm Hg	air mm Hg	△ mm Hg	
6	7.367	43.8	43.5	+ 0.3	Venous blood from subjects at rest
6	7.340	43.4	43.2	+ 0.2	Arterial or venous blood. Work intensity 900 mkg per minute. Air breathing.
8	7.266	43.8	43.5	+ 0.3	Arterial or venous blood. Work intensity 900 mkg per minute. 12.5 per cent O_2 in inspired air.
4	7.447	32.4	32.7	− 0.3	Venous blood from subjects at rest.
24				+ 0.2	

taken from 4 subjects under different experimental conditions are shown in table 1. It is seen that the pCO_2 measured indirectly by analyzing the blood in all cases is found to be nearly the same as the pCO_2 measured directly by analyzing the gas mixture in the tonometers. The mean difference between the indirect and direct carbon dioxide tensions for all experiments is + 0.2 mm Hg with a standard deviation of ± 0.68 and a standard error of ± 0.16. Similar experiments have been performed by HICKAM, PRYOR, PAGE and ATWELL (1951) who in 23 comparisons found an average difference between indirect and direct tensions of ± 1.62 mm Hg (standard deviation 2.03 and standard error 0.42) and by LAMBERTSEN, KOUGH, COOPER, EMMEL, LOESCHKE and SCHMIDT (1953) who found an average difference of + 3 mm Hg. The reason for the differences between the results of these three series of investigations is not clear.

The body temperature (rectal temperature) was in the work experiments measured during the period of air collection by means of a thermocouple, and in the rest experiments after the end of the experiments by means of a mercury thermometer.

The dead space was calculated by a rearrangement of BOHR's formula:

$$\text{Personal dead space} = \text{RD}\,\frac{(a-e)}{a} - \text{apparatus dead space}$$

where RD is respiratory depth, a is pCO_2 of arterial blood (replacing pCO_2 of alveolar expired air) and e is pCO_2 of expired air.

As subjects served 4 young male students (see table 2). The experiments were performed in the morning, the rest experiments after

Table 2.

Characteristics of subjects.

Subject	Height	Weight	Age	Vital capacity	Dead space at rest (BTPS)		
					Observed	Number of determinations	From Lindhard's formula
	cm	kg	years	liters	cc		cc
O. W.	188	81	20	6.13	196	4	181
S. L.	176	68	23	5.89	173	2	172
H. S.	180	67	21	5.33	158	6	168
M. L.	177	77	22	5.86	157	8	143

the subjects had rested for half an hour to three quarters of an hour, and the work experiments in the steady state of the work period after the subjects had worked for about fifteen minutes. After preliminary periods of suitable lengths during which the subjects breathed through the mouthpiece expired air and alveolar air were collected for about five minutes in the rest experiments and for about two to three minutes in the work experiments. The arterial blood was withdrawn at a slow and even rate through an indwelling needle which earlier had been introduced into the brachial artery. In the rest experiments, the time for collection of arterial blood did not cover the whole period of collection of expired air and alveolar air. No disturbance, however, was caused by the withdrawal of the blood, the subjects being all well trained in respiration experiments.

Results.

In table 2 are presented the average volume of the personal dead space from the rest experiments together with values calculated from the formula of LINDHARD (1914). By means of LINDHARD's formula, which is based on the relation between the trunk length and the dead space as measured by the hydrogen method of KROGH and LINDHARD, approximate values of the dead space can be obtained. Table 2 shows a quite good agreement between the observed and the calculated dead space values, the average of the latter being only slightly less than the average of the former.

The work experiments were carried out at the intensities of 360, 720 and 1,080 mkg per minute. The experiment with the highest work intensity, however, was performed only on one of the subjects (subject O. W.). In 29 of the work experiments

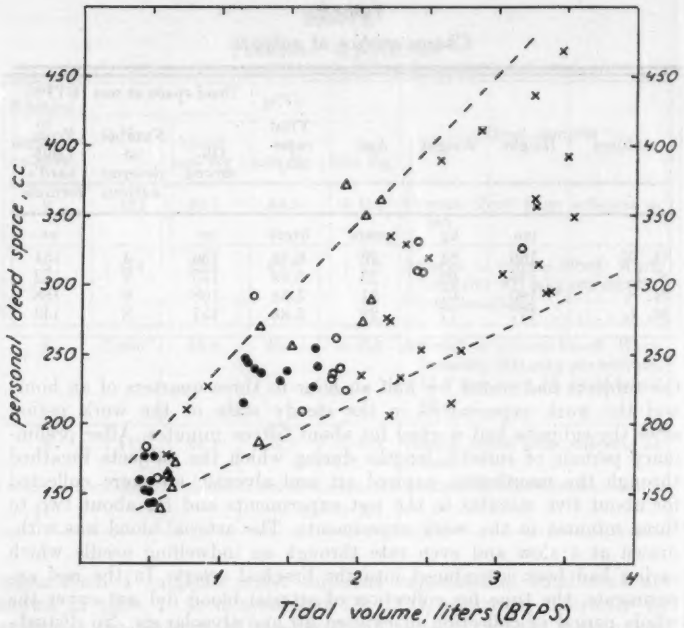

Fig. 1. Respiratory dead space (BTPS) in relation to tidal volume (BTPS) in rest and work experiments on 4 subjects.
x subject O. W., ● subject M. L., △ subject H. S. and ○ subject S. L.
The dotted lines are drawn so as to enclose practically all values.

the subjects were breathing air and in 22 of the experiments they were breathing 12.3 to 12.6 per cent oxygen in nitrogen.

In fig. 1 the determinations of the dead space (excluding the dead space of the apparatus) from both the rest and the work experiments are plotted against the tidal volumes. It is seen that a distinct correlation exists between the tidal volumes and the observed values of the dead space. Fig. 2 shows the relation between the averages of the observed values of the dead space and the tidal volumes. The rest value is calculated as the average of the four mean values in table 1, and the work values are calculated as the average of the single values measured at tidal volumes between 1 to 2, 2 to 3 and 3 to 4 liters, respectively. It is seen from fig. 2 that the dead space during muscular work shows a regular increase from the rest value of about 170 cc to a

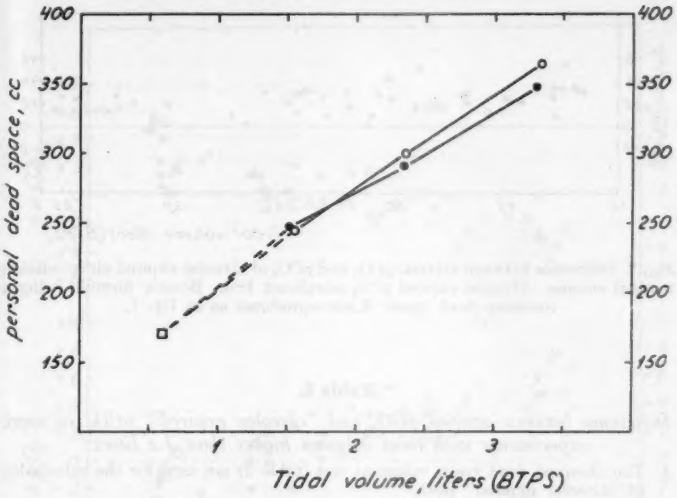

Fig. 2. Average values of respiratory dead space for 4 subjects in relation to tidal volume.

□ Rest experiments,

● Work experiments in atmospheric air, ○ Work experiments at low oxygen (12.2 to 12.8 % O_2 in inspired air, arterial HbO_2 % about 70).

value of 350 to 360 cc at a tidal volume of 3.3 liters. Further it is seen from fig. 2 that for the same tidal volume the dead space is practically the same when breathing low oxygen as when breathing air.

The scatter of the determinations in fig. 1 seems to be quite large. For the calculation of the composition of the alveolar air, however, even a considerable error in the dead space will be of only small influence if the tidal volume is large. If the values of the dead space represented by the lower dashed line in fig. 1 were used for the calculation instead of the average values in fig. 2 the error would for an alveolar pCO_2 of 40 mm amount to only — 1.0 mm Hg at a tidal volume of 3.3 liters and to — 1.6 mm Hg at a tidal volume of 1.5 liters. By using the dead space values represented by the upper dashed line in fig. 2 the error would be + 1.7 mm Hg at tidal volumes of both 3.3 and 1.5 liters. It must therefore be assumed that for subjects as those studied in the present investigation the alveolar (arterial) pCO_2 during deep breathing (tidal volume about 1.5 liters or above)

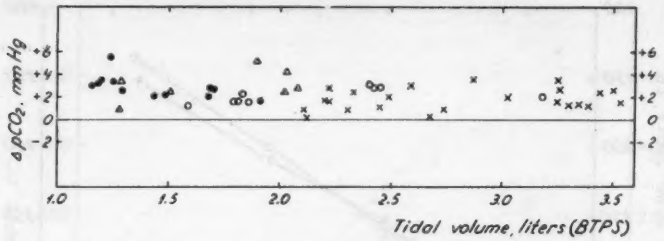

Fig. 3. Difference between arterial pCO_2 and pCO_2 of alveolar expired air in relation to tidal volume. Alveolar expired pCO_2 calculated from BOHR's formula using a constant dead space. Same signatures as in fig. 1.

Table 3.

Difference between arterial pCO_2 and "alveolar expired" pCO_2 in work experiments with tidal volumes higher than 1.4 liters.

A: The observed dead space values at rest (table 2) are used for the calculation of "alveolar expired" pCO_2.

B. The dead space values calculated from Lindhard's formula (table 2) are used for the calculation of "alveolar expired" pCO_2.

Subject	Number of determinations	A		B	
		Difference	S. D.	Difference	S. D.
M. L.	6	2.2	± 0.4	2.6	± 0.4
O. W.	23	1.8	± 0.9	2.0	± 0.9
S. L.	9	2.1	± 0.7	2.1	± 0.7
H. S.	5	3.4	± 1.2	3.2	± 1.2
	45	2.1	± 1.0	2.2	± 1.0

in muscular exercise can be determined with a considerable degree of accuracy when using the average values of the dead space in fig. 2.

In fig. 3 and table 3 the arterial pCO_2 values that were used for the calculation of the dead space values in fig. 1 are compared to the corresponding "alveolar expired pCO_2" values, as determined by BOHR's formula, using the observed resting values of the dead space from table 2. As mentioned on page 2 it is a genera practice to calculate the composition of the alveolar expired air in work experiments, by using a constant value (the rest value) of the dead space. Fig. 3 and table 3 show that the difference between arterial pCO_2 and "alveolar expired pCO_2" is nearly the

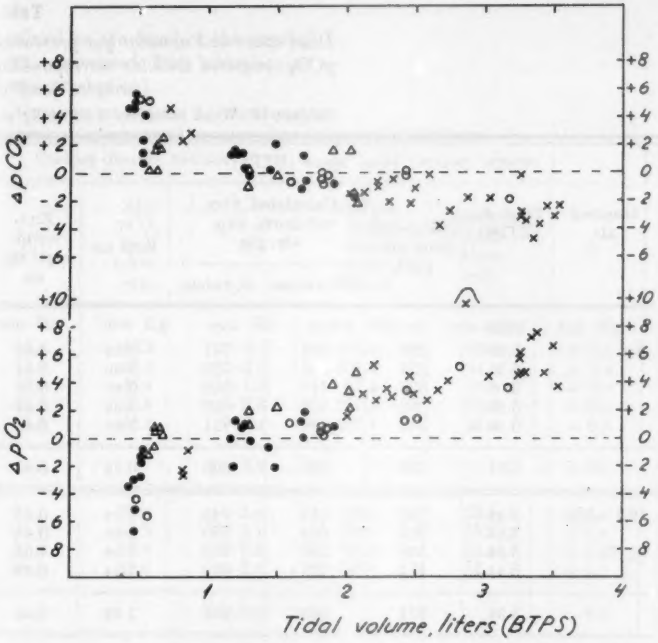

Fig. 4. *Upper curve:* Difference between arterial pCO_2 and alveolar pCO_2 (end-normal expiration method) at different tidal volumes. *Lower curve:* Difference between alveolar pO_2 as calculated from BOHR's formula using the physiological dead space for CO_2 and end-normal expired pO_2 at different tidal volumes. Signatures as in fig. 1.

same for all subjects and that it is nearly independent of the work intensity (360 to 1,080 mkg per minute) and the tidal volume. For determinations at tidal volumes above 1.4 liters the difference is about 2 mm Hg for 3 of the subjects and 3.4 mm Hg for the fourth subject. The average difference of all determinations above tidal volumes of 1.4 liters is 2.1 mm Hg with a standard deviation of 1.0 mm Hg. If the dead space values calculated from LINDHARD's formula (table 2) are used instead of the observed values the average difference between arterial pCO_2 and alveolar expired pCO_2 is 2.2 mm Hg with a standard deviation of 1.0 mm Hg.

In fig. 4 the indirectly (from the blood samples) determined alveolar gas pressures are compared to the alveolar gas pressures

Table

Dead space and alveolar gas pressures, pCO_2 compared with the corresponding samples (end of

Subject W. Work intensity 1 080 mkg per

Inspired air	Tidal air (BTPS)	Dead space (BTPS)			RQ	
		Calculated from art. pCO_2	Calculated from end-norm. exp. alv. gas		Exp. air	End-norm. exp. alv. air
			CO_2 values	O_2 values		
	liters	cc	cc	cc		
Air	3.39	296	514	741	0.89	0.80
	3.35	294	582	736	0.90	0.84
	3.03	308	410	579	0.89	0.84
	3.26	362	522	669	0.89	0.84
	3.50	392	598	751	0.90	0.85
	3.31	330	525	695	0.89	0.83
12.1—12.3	3.30	316	615	748	1.02	0.96
	3.25	357	624	730	1.00	0.96
% O_2	3.54	348	569	718	1.06	1.00
	3.44	474	705	812	1.04	0.99
	3.38	374	628	752	1.03	0.98

as determined by direct sampling (end-normal expiration method). The indirectly determined alveolar pO_2 which in normal man probably is close to the mean alveolar pO_2 (see discussion), is in the work experiments calculated from BOHR's formula by use of the dead space values from the curves in fig. 2; in the rest experiments the average of the observed values for each subject (table 2) is used for the calculation. The upper curve in fig. 4 shows the difference between arterial (alveolar) pCO_2 and pCO_2 of the end-normal expired alveolar air, and the lower curve shows the difference between the indirectly determined alveolar pO_2 and the pO_2 of the end-normal expired alveolar air. It is seen from fig. 4 that at small tidal volumes (0.4 to 0.5 liters) the arterial pCO_2 is higher and the indirectly determined alveolar pO_2 lower than the corresponding values of the "end-normal expired" alveolar gas pressures. At the large tidal volumes during muscular work (about 2 to 3.5 liters) the arterial pCO_2 conversely is lower and the indirectly determined alveolar pO_2 higher than the

4.

calculated on the basis of the arterial values, calculated from alveolar air normal expiration).

min. Oxygen consumption 2.5 liters per min.

Carbon dioxide tension			Oxygen tension		
Art. pCO_2	Alv. pCO_2 End-norm. exp.	Δ	Alv. pO_2 Indirectly determined	Alv. pO_2 End-norm. exp.	Δ
mm Hg	mm Hg	mm Hg	mm Hg	mm Hg	mm Hg
44.5	48.2	— 3.7	100.2	92.8	+ 7.4
43.9	48.6	— 4.7	101.6	94.3	+ 7.3
46.6	48.4	— 1.8	97.8	93.8	+ 4.0
43.1	45.9	— 2.8	101.3	95.3	+ 6.0
43.1	46.2	— 3.1	102.1	95.3	+ 6.8
		— 3.2			+ 6.3
30.2	33.6	— 3.4	59.8	55.1	+ 4.7
30.2	33.3	— 3.1	59.7	55.1	+ 4.6
30.6	32.9	— 2.3	56.5	52.6	+ 3.9
28.6	31.0	— 2.4	58.8	54.1	+ 4.7
		— 2.8			+ 4.5

corresponding "end-normal expiration" values of the alveolar gas pressures. This is also seen from the values in table 4 which shows the results from the work intensity of 1,080 mkg per minute. Table 4 furthermore shows, that the dead space values calculated on the basis of the end-normal expired alveolar pCO_2 is considerably higher than the values calculated by use of the arterial pCO_2 and that the dead space values calculated on the basis of end-normal expired alveolar pO_2 are still higher. In parallel to the differences between the CO_2 and the O_2 values of the dead space, corresponding differences are found between the RQ of the expired air and the RQ of the end-normal expired alveolar gas.

Discussion.

In the present study it was found, that the dead space determined by use of the arterial pCO_2, usually termed the physiological dead space, increased from an average value of 170 cc during

rest to 350 cc to 360 cc during muscular work in which the tidal volume reached a value of 3.3 liters. The ratio of the dead space to tidal volume consequently decreased from 0.28 to 0.11. This is in contrast to the findings of ENGHOFF (1938) and ROSSIER and BÜHLMANN (1950) which show a constant ratio of about 0.3, but is in agreement with the results of RILEY et al. (1954), COHN et al. (1954) and FILLEY et al. (1954). None of these authors found a clear correlation between dead space and tidal volume during exercise but a calculation of mean values from their data shows that for corresponding tidal volumes the size of the dead space found by RILEY, COHN and co-workers was about the same as found in the present investigation, whereas FILLEY et al. (1954) found it somewhat higher although the difference is not large. The conclusions of ENGHOFF are based on but a few experiments while those of ROSSIER and BÜHLMANN apparently are based on a large material. The determinations of the last mentioned authors pre-suppose a complete absorption of the produced CO_2 in their closed system. It is possible that this demand has not been fulfilled in the work experiments and all errors deriving from this technical default will tend to increase the calculated size of the dead space.

The increase in physiological dead space found in the present investigation during muscular work with increasing tidal volumes is somewhat larger than the increase in the so-called "anatomical" dead space as determined by KROGH and LINDHARD (1917), MUNDT, SCHOEDEL and SCHWARZ (1941), FOWLER (1948), PAPPENHEIMER, FISHMAN and BORRERO (1952) and YOUNG (1955). In the present investigation the increase was found to be almost 200 cc, whereas KROGH and LINDHARD both in rest and work found an increase of only 100 cc at *maximal* inflation of the lungs, and the increase found by the other authors was of about the same magnitude. The "anatomical" dead space can be defined as the volume of pure unmixed inspiratory air contained in the airways at the end of an inspiration. It could be assumed that the increase of this volume with increasing inflation of the lungs should equal the increase in capacity of the non respiratory part of the airways, *i. a.* trachea, bronchi etc. Calculations by FOWLER based on anatomical and roentgenological studies (ROHRER 1915 and HUIZINGA 1936) show that this capacity can increase about 230 cc by maximal inflation of the lungs. The smaller increase found by gas analytical methods may be explained as being due

to an admixture of alveolar air to dead space air caused by diffusion or by mechanical agitation of the gases (FOWLER 1948, DUBOIS, FOWLER, SOFFER and FENN 1951, BARTELS et al. 1954, and ROOS, DAHLSTRÖM and MURPHY 1955).

As mentioned before the difference between the arterial pCO_2 which formed the basis for the increasing dead space found in work, and the alveolar pCO_2 determined from BOHR's formula under the assumption of a constant dead space (the rest value) during work is about 2 mm Hg (range 1.8 to 3.4) for the four subjects investigated (fig. 3 and table 3). This difference would have been somewhat smaller if the increase in the "anatomical" dead space which is caused by the increased inflation of the lungs had been taken into account in the calculation of the alveolar pCO_2. But even with this reservation there would probably have been a difference between the arterial pCO_2 and the alveolar pCO_2 calculated as above. This difference might have been caused by a systematic technical error in the determination of the arterial pCO_2 but this is less probable since the procedure has been checked by tonometric determinations. A possible reason for the difference between the arterial pCO_2 and the calculated alveolar pCO_2 might be that a diffusion gradient exists across the alveolar wall. It is generally accepted that at rest an equilibrium is reached between alveolar and arterial pCO_2 but even so a small gradient might be found during muscular work when the CO_2 production is large. The fact that the found difference between arterial and calculated alveolar pCO_2 does not increase with increasing work intensity, however, makes the assumption that the difference is due to such a gradient unlikely.

One reason for the difference between arterial pCO_2 and calculated pCO_2 shown in fig. 3 is as pointed out by KROGH (1913) and mentioned on page 2 that even with a completely uniform ventilation and perfusion of different parts of the lungs a small difference must exist between the mean alveolar gas tensions and the gas tensions of the "alveolar expired" air as calculated from the BOHR formula. Further it is possible that even in normal subjects slight inequalities in ventilation-perfusion ratios exist in different parts of the lungs, and if this is so it will contribute to the difference between arterial and "alveolar expired" pCO_2 and hence on the difference between "physiological" and "anatomical" dead space.

The influence of unequal ventilation and perfusion of different parts of the lungs and its influence on the alveolar gas tensions and the dead space has been discussed *e. g.* by MUNDT, SCHOEDEL and SCHWARZ (1941), RILEY and CORNAND (1949), RAHN (1949), BANNISTER, CUNNINGHAM and DOUGLAS (1954) and FOLKOW and PAPPENHEIMER (1955).

Whereas the alveolar pCO_2 as calculated from the BOHR formula by use of the "anatomical" dead space was found to be lower than the arterial pCO_2 the alveolar pCO_2 determined by direct sampling of alveolar air (end-normal expiration method) was found to be higher than the arterial pCO_2 in heavy work with tidal volumes of about 1.5 liters or higher (fig. 4 and table 4). SUSKIND et al. (1950), FILLEY et al. (1954) and BARTELS et al. (1954) also found that the end-tidal pCO_2 during work is higher than the arterial pCO_2. The dead space calculated on the basis of end-tidal pCO_2 is consequently somewhat higher than when calculated by use of the arterial pCO_2 (table 4). The values are nearly the same as those found by BANNISTER et al. (1954) from end-tidal air. The reason why the end-tidal pCO_2 is higher than the arterial pCO_2 is probably that the alveolar samples represent the last part of the alveolar expired air, which during work undergoes large changes during the respiratory cycle (DUBOIS et al. 1952 and YOUNG 1955). The end-tidal pCO_2 will, therefore, be too high and the end-tidal pO_2, too low, but the error on the pCO_2 will be the smaller because the liberation of CO_2 from the blood will be slowed down on account of the increasing concentration of CO_2 in the alveoli during the expiration. As a consequence the RQ of the end tidal air will be lower than that of the expired air and the error on the dead space calculated from the end-tidal pCO_2 will be smaller than when the dead space is calculated from the end-tidal pO_2 (table 4). The difference between end-tidal pCO_2 and arterial pCO_2 and also between RQ of expired and end-tidal air will be aggravated if hypoventilated parts of the lungs preferably empty themselves late in the expiration.

The fact that nearly the same difference between the RQ of end-tidal and of expired air exists in the experiments with low oxygen (table 4) indicates that the slowing-down of the CO_2 elimination from the blood in the course of an expiration also in this condition (end-tidal pO_2 about 55 mm Hg) is more pronounced than the concomitant slowing down of the oxygen uptake which probably takes place at the low alveolar pO_2. This is in agreement

with the findings of OTIS, RAHN and FENN (1948) who found that the RQ during breath holding decreased even at an alveolar pO_2 of 36 mm Hg at the end of the breath holding period.

While the difference between the end-tidal pCO_2 and the arterial pCO_2 in the heavy work experiments (table 4) is only about 3 mm Hg the difference between the pO_2 of the end-tidal air and the pO_2 of the alveolar air calculated from the BOHR formula under the assumption that the dead space for O_2 is equal to the dead space for CO_2 is somewhat larger (4.5 to 6.3 mm Hg). This is a consequence of the above mentioned circumstance that the error on the end-tidal pCO_2 is smaller than the error on the end-tidal pO_2.

The assumption that the dead space for O_2 is equal to the dead space for CO_2 can be expressed by the equation

$$\frac{RD\ (e_{O_2} - a_{O_2})}{i_{O_2} - a_{O_2}} = \frac{RD\ (a_{CO_2} - e_{CO_2})}{a_{CO_2}}$$

where RD is respiratory depth and a, e and i denote fractions or tensions of the gases in alveolar, expired and inspired air. By rearranging and solving for a_{O_2} one gets

$$a_{O_2} = i_{O_2} - \frac{a_{CO_2}\ (i_{O_2} - e_{O_2})}{e_{CO_2}}$$

This latter expression can be shown to be another form of the "alveolar air equation" of FENN, RAHN and OTIS (1946). It is consequently the same whether the alveolar pO_2 is calculated from BOHR's formula by using the physiological dead space for CO_2 or whether it is calculated from the arterial pCO_2 and the RQ of the expired air using the "alveolar air equation" as done by RILEY and COURNAND (1949).

Summary.

The respiratory dead space (physiological dead space) calculated from the BOHR formula on the basis of arterial pCO_2 has been determined on 4 young healthy subjects at rest and during light to heavy muscular work.

It was found that the physiological dead space during work increased with the tidal volume from an average value of about 170 cc at rest to about 350 cc during work at a tidal volume of about 3.3 liters. The values were nearly the same when breathing at-

mospheric air and when breathing air with a low oxygen content (about 12.5 % O_2 in inspired air).

In muscular work with tidal volumes higher than about 1.5 liters the arterial pCO_2 was found to be 2 to 3 mm Hg *higher* than the alveolar pCO_2 calculated from the BOHR formula using a constant dead space at modum KROGH and LINDHARD. The arterial pCO_2 was on the other hand in heavy work found to be about 3 mm Hg *lower* than the alveolar pCO_2 determined by direct sampling (end-normal expiration method). The alveolar pO_2 determined by direct sampling was in heavy work found to be about 6 mm Hg lower than the alveolar pO_2 calculated from BOHR's formula using the physiological dead space for CO_2. The possible reasons for the differences are discussed in the text.

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From the Dept. of Physiology, Kungl. Veterinärhögskolan, Stockholm.

Water and Food Intake and the Inhibitory Effect of Amphetamine on Drinking and Eating before and after "Prefrontal Lobotomy" in Dogs.

By

B. ANDERSSON and S. LARSSON.

Received 10 July 1956.

Beside lesions of the hypothalamus it has also been shown that destruction of the connections between the frontal lobes and the thalamus may lead to increased appetite in man (COBB 1944), but conclusive evidence for changes in water metabolism after prefrontal lobotomy has not been given.

Amphetamine is known to depress appetite in man (NATHANSON 1937, DAVIDOFF and REIFENSTEIN 1937, ULRICH 1937) and in the dog (ALPERN, FINKELSTEIN and GANTT 1941). HARRIS, IVY and SEARLE (1947) have given evidence that patients with frontal lobotomy are less susceptible to the anorexic effect of amphetamine than are normal persons. The influence of this drug on thirst does, however, not seem to have been studied. Having this in mind the following experiments in dogs were undertaken.

Methods.

Eleven dogs of different breeds were used for the experiments. The animals were fed a standard diet beeing a mixture of 80 % meat and 20 % mashed dog crackers once a day between 15.00 and 15.30 o'clock. Five of the dogs were given this standard diet ad libitum and the others received 300 g of the diet per 10 kg of body weight and day. All the animals had free access to water and their water intake was continuously followed. In some cases urine volume and urinary sodium, potassium and chloride outputs were measured.

A repeatable "thirst" stimulus of constant intensity in one and the same animal was obtained by the intravenous injection of 20 cc of a

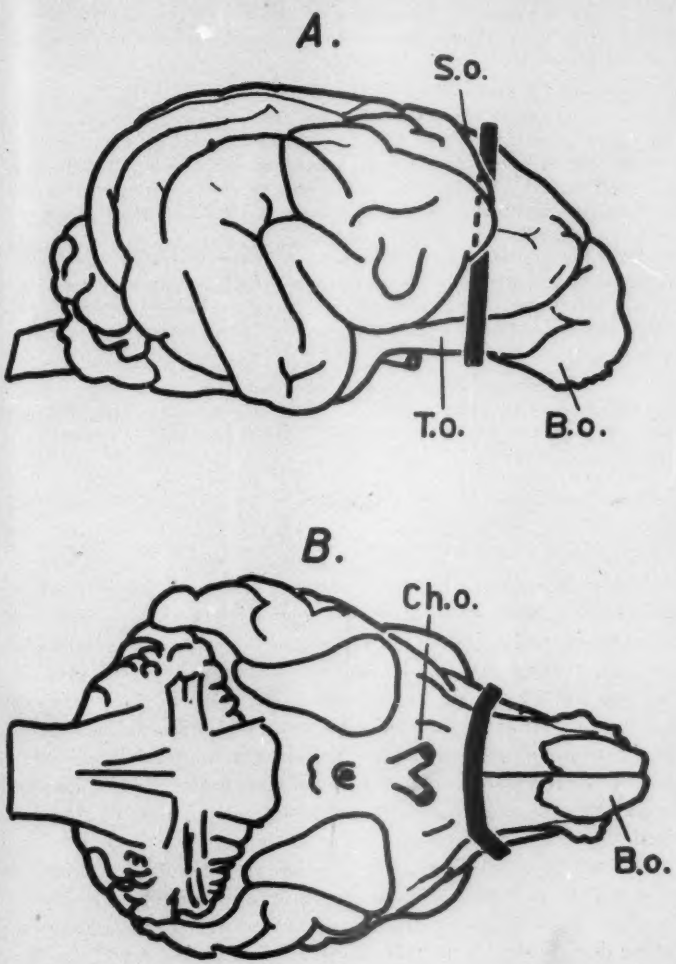

Fig. 1. Lateral (A.) and ventral (B.) views of the brain of a dog showing the direction of the sections made and the approximate extent of the part of the forebrain separated by a complete section.

B. o.: Bulbus olfactorius.
Ch. o.: Chiasma opticum.
S. o.: Sulcus orbitalis.
T. o.: Tractus olfactorius.

20 % NaCl solution. Amphetamine (Phenopromin ACO) was administered by intramuscular injection one hour before feeding or application of the "thirst" stimulus.

Operations. A more or less complete interruption of the connections between the "prefrontal lobes" and the rest of the brain was obtained by sucking out the nervous tissue bilaterally through and just anterior to the orbital sulcus in a direction passing well anterior to the caput of the caudate nucleus. The brain was hereby approached through the frontal sinus under nembutal anaesthesia. The recovery of the animals after this operation was fast.

After the termination of the experiments the dogs were sacrified by an intravenous injection of nembutal and the heads were perfused with BOUIN's fixative. After fixation and paraffine imbedding the brains were serially sectioned in order to determine the extent of the lesions made.

Chemical methods: Sodium and potassium analyses in plasma and urine were made using the EEL flame photometer. Urine chloride was determined according to the Volhard method (PEETERS and VAN SLYKE 1932).

Results.

A. Water metabolism and food intake before and after operation.

The group of dogs *kept on a restricted diet* continued to eat all the food offered even after the operation and no significant changes in water intake, urine volume or urinary excretion of sodium, potassium and chloride were observed. Tests with injections of hypertonic saline were not made in this group of animals. In one of the dogs the separation of the frontal pole of the brain was complete, thus also including a bilateral interruption of the olfactory tracts. In the other five animals the olfactory tracts and some on the ventral and lateral portions of the forebrain were left untouched by the section.

In the group of dogs *fed ad libitum* the operation resulted in a complete section through the forebrain, including an interruption of the olfactory tracts, in three of the animals. The two other dogs belonging to this group showed an incomplete section leaving the olfactory tracts and some of the ventral part of the forebrain untouched. Fig. 1 shows the direction of the sections made and approximately the extent of the part of the forebrain separated by a complete section.

One of the ad libitum fed dogs had a very high food intake during the $2^1/_2$ months long control period before the operation (average 1,650 g per day). During this period of time its body

weight increased from 16.5 kg to 22.3 kg at the time of operation when the animal was slightly obese. After a complete section of the forebrain including the olfactory tracts this dog reduced its food intake to a more "normal" level (average 800 g per day) and lost 2 kg of weight during the month it was observed after the operation. The two other dogs with complete section of the forebrain only showed a slight postoperative reduction of food intake. These two animals had, however, not shown notably high food intake before the section through the forebrain was made.

The animals which reduced their food intake after the operation also drank less water than before, the ratio of water to food being unchanged or slightly elevated after the operation. None of the dogs with a section through the forebrain not extending further caudally than to the plane shown in Fig. 1 did respond with less drinking to an intravenous injection of 20 cc of a 20 % NaCl solution than they had done during the control period before the operation. This was on the other hand the case in another animal where the lesion had purposely been extended ventro-caudally to involve parts of the preoptic area on both sides. After this lesion that dog, which before the operation had drunk about 300 cc of water subsequent to each "thirst" stimulus, did no longer, when tested on four occasions during the first four weeks after the operation, respond with drinking of water to the "thirst" stimulus used. During this time, however, it ate the rations offered and drank during the five hours following upon the food intake about the same amount of water it had drunk daily before the operation and showed, although a slight increase in plasma sodium concentration was observed, no obvious signs of dehydration. Six weeks after the operation a renewed injection of hypertonic saline was again effective in causing the animal to drink almost the same amount of water as it had drunk under similar conditions before the operation.

B. The effect of amphetamine on drinking and eating before and after the operation.

As could be expected from previous observations (ALPERN, FINKELSTEIN and GANTT 1941, HARRIS, IVY and SEARLE 1947) the intramuscular administration of 1 to 1.5 mg of amphetamine per kg body weight one hour before feeding completely inhibited or markedly reduced the food intake in all animals before the

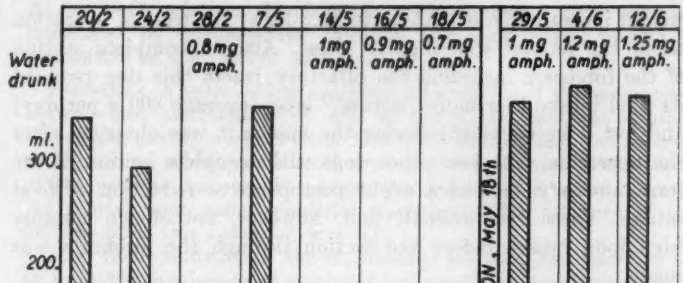

Fig. 2. A graph showing the water intake consequent to repeated "thirst" stimuli in a dog before and after a complete section through the forebrain. The intravenous injection of 20 cc of a 20 % NaCl solution was used as "thirst" stimulus. Where amphetamine was administered one hour prior to the intravenous injection of hypertonic saline the dose per kg body weight is indicated on the graph.

Hatched columns: The amount of water drunk within 30 minutes after the intravenous injection of hypertonic saline.

White columns: The amount of water drunk between 30 and 90 minutes after the injection.

operation. Since, however, the simultaneous absence of drinking could not be taken as a clear evidence for an inhibitory effect of the drug also on the urge to drink, tests with intravenous injections of hypertonic saline were made in five of the dogs. After an intravenous injection of 20 cc of a 20 % NaCl solution the animals drank between 250 and 400 cc of water during half to one hour following the injection. The amount of water drunk after such a test was fairly constant in each separate dog. If, however, 1.0 mg of amphetamine was given one hour before the injection of hypertonic saline all the animals tested but one did not drink at all during the hour following the saline injection. In the exceptional animal the drinking was delayed and the water intake was only half the amount of that the animal drank on a similar test where no previous administration of amphetamine had been made.

In three of four animals tested a complete section through the forebrain made in the direction shown in Fig. 1 had the effect

that a dose of amphetamine which before the operation had inhibited the drinking after an intravenous injection of hypertonic saline now no longer had this effect. Fig. 2 shows the different "thirst" tests made in one of these animals before and after a section had been made through the forebrain.

A diminished inhibitory effect of amphetamine on the food intake of the animals was less consistently observed after the operation. Thus those ad libitum fed dogs, in which the separation of the frontal pole of the brain was complete including a bilateral interruption of the olfactory tracts and which showed a more or less reduced food intake after the operation, did not show any decreased sensitivity to the drug in respect to its inhibitory effect on food intake. But in 5 out of 8 animals with lesions not interrupting the olfactory tracts and leaving more or less of the ventral and lateral parts of the forebrain untouched, the dose of amphetamine needed to inhibit food intake completely was 50 to 100 % larger after the operation than it had been during the control period before.

Discussion.

There is little if any evidence that the prefrontal areas of the cerebral cortex should be of any primary importance in the regulation of water and electrolyte metabolism. Thus STEVENSON and MUNSON (1950) found no metabolic changes of this kind in humans after undercutting of areas 8 and 9. An elevation of plasma sodium and chloride concentrations has further only rarely been observed after frontal lobotomy in man and was then seen in combination with other changes of metabolism (SWEET, COTZIAS, SEED and YAKOVLEV, 1947).

The experiments described here indicate that an isolation of the frontal pole of the brain in dogs anterior to the plane shown in Fig. 1 does not diminish the conscious urge to drink or cause any other significant changes in the water and electrolyte metabolism of the animals. The only dog in this series of experiments which obviously showed such changes after the operation was an animal where the lesion had been extended ventro-caudally to involve parts of the proptic area. Here the absence of a drinking response to intravenous injections of strongly hypertonic saline during one month after the operation seemed to have been due to a temporary impairment of the animal's osmoregula-

tion resulting in a lack of any stronger conscious urge to drink in spite of considerable elevation of the extracellular osmotic pressure. The urge to drink following the ingestion of food did, however, seem to be unaffected by this lesion. ANDERSSON and MCCANN (1955) observed that electrical stimulations of the anterior part of the "drinking area" of the hypothalamus of the goat also caused an inhibition of water diuresis of neurohypophyseal type, whereas that was not the case when the posterior parts of this area was stimulated. Hyperphagia could there, on the other hand, occasionally be seen in combination with the polydipsia during the stimulations. CORT (1951) in a study of the interrelationship of hunger and thirst found a significant rise of serum sodium concentration in rats made obese by lesions medially in the hypothalamus when the determinations were made without control of time and amount of food intake. When, however, analyses were done six hours after the ingestion of a standard meal there was no difference in serum electrolyte concentrations between lesion and control rats. In view of these observations the changes in water metabolism seen in the above-mentioned dog may be looked upon as an indication of a dualism of the hypothalamic "thirst centre", in which an osmotic stimulus and a thirst stimulus of other kind — somehow related to satiety or to the mechanism of food absorption — are acting on different neurons.

In three out of five ad libitum fed dogs a complete section of the forebrain, including an interruption of the olfactory tracts, resulted in a more or less pronounced decrease in food intake. This decrease may, however, not have been the consequence of an isolation of parts of the prefrontal cortex of importance for the regulation of food intake, but perhaps more likely have depended on the postoperative disappearance of smell sensations stimulating the appetite of the dogs.

BROBECK, LARSSON and REYES recently (1956) made a study of the effect of amphetamine on the activity in the hypothalamic centre regulating food intake and found that the drug gives rise to an increased electrical activity in the ventromedial hypothalamic nuclei of the cat. They interpreted the results as indicating that amphetamine inhibits the urge to eat by causing an increased activity in the "satiety centre", localized to the ventromedial hypothalamic nuclei, either through a direct stimulatory action on neurons in these nuclei or indirectly from

structures in the frontal lobes or other association areas of the cerebral cortex. The results of the experiments reported here show that amphetamine does not specifically inhibit the urge to eat but also — and perhaps more pronounced — the urge to drink in the dog, possibly by an indirect or direct inhibitory effect on the hypothalamic "thirst centre". The experiments further indicate that — whatever the mechanism of the inhibitory action of amphetamine on the urges to eat and drink may be — this inhibitory effect is at least partly excerted from structures localised to the prefrontal areas of the cerebral cortex.

Summary.

1) A complete section through the forebrain through and just anterior to the orbital sulcus and directed well anterior to the caput of the caudate nucleus was not seen to primarily reduce the water intake of dogs or to cause any reduction in the amount of water drunk by the animals subsequent to the intravenous injection of a certain amount of hypertonic saline. The latter was temporarily the case in one animal where the lesion was extended in ventro-caudal direction to involve the preoptic area on both sides.

2) Amphetamine (1 mg per kg of body weight) completely inhibited the drinking of water after an intravenous injection of 20 cc of a 20 % NaCl solution in 4 of 5 normal dogs tested. This was no longer the case in 3 of these animals after "prefrontal lobotomy".

3) An incomplete section through the forebrain in 5 out of 8 dogs made that the dose of amphetamine necessary to inhibit the food intake of the animals completely had to be increased by 50 to 100 %.

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From the Department of Physiology, University of Aarhus.

The Influence of Different Ions on the Intestinal Absorption of Sodium and Chloride.

By

SV. E. BUDOLFSEN.

Received 11 July 1956.

Previously (BUDOLFSEN 1955) it was observed that the sodium as well as the chloride ions are absorbed against a concentration gradient in the colon of rats when the ions are infused as a sodium chloride solution. In some experiments the same phenomenon could be observed in the ileum when a hypotonic solution of sodium chloride was infused, because the normally occurring increase in concentration did not have such an intensity that the blood concentration of sodium or chloride was reached.

In this paper it has been the purpose to investigate the influence of different anions and cations on the intestinal absorption of sodium and chloride ions.

Experiments.

The experiments were made on white rats with the same technics as previously described (BUDOLFSEN 1955). The infused volume in all experiments was 2 ml, and the absorption time 60 minutes.

Table I shows the chloride net-absorption from some different chloride solutions. The average weight of the animals in the different series varies a little, so that it is most correct to take the percentage net-absorption as an expression of the net-absorption. The percentage net-absorption is here the absorption in per

Table I.

Table I shows the influence of different cations on the chloride net-absorption. Infused volume 2 ml, absorption time 60 minutes.

Solution infused	Cl %	Colon			Small Intestine		
		absorbed pr. 100 g animal mg	absorbed in %	Concentr. at the end in %	absorbed pr. 100 g animal mg	absorbed in %	Concentr. at the end in %
NH_4Cl	0.55	4.00	69.1	0.159	4.90	86.1	0.279
—	—	5.60	73.8	0.176	5.90	78.2	0.292
—	—	3.07	53.9	0.251	2.15	37.7	0.527
—	—	3.83	70.8	0.205	2.65	49.0	0.488
—	—	3.33	62.2	0.201	3.71	69.5	0.442
NH_4Cl average		3.97	66.0	0.198	3.86	64.1	0.406
KCl	0.55	3.10	46.4	0.270	3.31	49.5	0.413
—	—	4.51	67.9	0.213	3.65	54.2	0.381
—	—	2.70	56.8	0.192	3.10	65.6	0.369
—	—	2.50	53.1	0.198	1.90	41.5	0.444
—	—	2.90	56.8	0.191	2.70	53.6	0.426
KCl average		3.14	56.2	0.213	2.93	52.9	0.407
$CaCl_2$	0.58	3.22	44.8	0.290	4.17	57.9	0.530
—	—	2.99	48.1	0.277	3.69	59.4	0.398
—	—	3.97	51.1	0.351	3.45	44.4	0.528
$CaCl_2$ average		3.39	48.0	0.306	3.77	53.9	0.485
$MgCl_2$	0.55	2.35	39.6	0.301	2.78	46.9	0.461
—	—	2.18	36.8	0.254	1.36	23.5	0.621
—	—	3.08	42.3	0.320	2.27	31.2	0.506
—	—	2.80	47.8	0.216	2.35	40.2	0.503
$MgCl_2$ average		2.6	41.6	0.273	2.19	35.5	0.523
NaCl	0.55	3.35	62.5	0.219	3.55	66.1	0.411
—	—	3.87	59.2	0.188	4.86	74.9	0.274
—	—	3.27	60.9	0.189	4.34	80.9	0.291
—	—	3.22	45.9	0.256	3.81	54.3	0.370
—	—	4.62	65.8	0.220	4.47	63.7	0.342
—	—	4.69	67.4	0.205	4.71	62.5	0.380
NaCl average		3.84	60.4	0.213	4.29	67.1	0.345

cent of the infused amount per 100 gram animal. The infused solutions have all a chloride concentration of about 0.55 per cent.

In the colon the chloride net-absorption decreases gradually in the following order: NH_4 — Na — K — Ca — Mg. The difference is

significant between the first- and the last-mentioned cation, but not between the neighbouring cations. In the ileum the following order was observed: $Na - NH_4 - Ca - K - Mg$, but here also a significant difference is seen only between the first- and the last-mentioned cations.

The chloride concentration of the intestinal content at the end of the experiments is also shown in Table I. The decrease in chloride concentration of all solutions is much higher in the colon than in the ileum — as was previously (BUDOLFSEN 1955) observed for sodium chloride solutions only. This seems to be caused by a much larger water net-absorption in the ileum than in the colon. In each intestinal segment the decrease in chloride concentrations seems to be higher, the higher the chloride net-absorption. On an average the chloride concentration of the blood was 0.328 % in 53 rats, so that the chloride concentration of the intestinal content decreases below that of the blood in all the colon experiments. This does not happen in the ileum. The difference between the two intestinal segments was found to be still more pronounced when using half isotonic solutions of KCl, NH_4Cl and NaCl — here a uniform decrease in the chloride concentration was observed during the absorption in the colon, while a uniform increase on concentration to about that of the blood was observed in the ileum.

Table II shows the sodium net-absorption when different solutions of sodium salts — all of the same concentration (0.213 %) — were infused. In the colon the sodium net-absorption is identical from solutions of $NaHCO_3$, NaCl, CH_3COON_2 and $NaNO_3$, while it is smaller from a solution of Na_2SO_4. In the ileum the sodium net-absorption is higher from solutions of NaCl and CH_3COON_2 than from $NaNO_3$ and $NaHCO_3$. The last-mentioned is not absorbed more quickly than Na_2SO_4, but there are only 3 experiments with $NaHCO_3$.

During the absorption of the different sodium salts in the colon the decrease of the sodium concentration of the intestinal content is the higher, the higher the sodium net-absorption, just as it was observed during the chloride absorption. In the ileum the sodium concentration of the intestinal content increases — the increase is the higher the smaller the net-absorption. In many experiments the sodium concentration of the intestinal content becomes smaller than that of the blood (on an average 0.308 % in 38 animals). In all the colon experiments it is thus possible to

Table II.

Table II shows the influence of different anions on the sodium net-absorption. Infused volume 2 ml, absorption time 60 minutes.

Solution infused	Na %	Colon			Small Intestine		
		absorbed pr. 100 g animal mg	absorbed in %	Concentr. at the end in %	absorbed pr. 100 g animal mg	absorbed in %	Concentr. at the end in %
$NaHCO_3$	0.213	1.41	51.5	0.148	0.86	31.4	0.378
—	—	1.07	41.6	0.136			
—	—	0.50	22.5	0.219	1.14	51.3	0.338
—	—	1.93	79.2	0.130	0.75	30.8	0.355
$NaHCO_3$ average		1.23	48.7	0.158	0.92	37.8	0.357
CH_3COONa	0.213	1.30	46.8	0.138	2.20	80.6	0.156
—	—	1.70	63.2	0.102	1.80	69.3	0.215
—	—	0.80	28.9	0.152	1.40	49.0	0.230
—	—	0.50	21.4	0.203	1.50	63.4	0.297
—	—	1.20	43.8	0.140	1.20	42.6	0.401
CH_3COONa average		1.10	40.8	0.147	1.62	61.0	0.260
$NaNO_3$	0.213	1.10	45.2	0.142	0.55	22.6	0.292
—	—	0.87	42.0	0.165	0.68	33.1	0.267
—	—	0.95	37.2	0.170	0.75	29.2	0.343
—	—	1.10	38.8	0.174	1.47	51.5	0.332
—	—	0.92	34.3	0.167	1.34	50.0	0.270
—	—	1.32	44.0	0.150	1.52	50.1	0.316
$NaNO_3$ average		1.04	40.3	0.161	1.05	39.4	0.303
Na_2SO_4	0.213	0.37	18.0	0.200	0.75	36.6	0.340
—	—	0.21	10.8	0.207	0.58	29.3	0.432
—	—	0.30	15.0	0.241	0.65	32.7	0.460
—	—	0.39	16.0	0.210	1.21	48.7	0.420
—	—	0.00	00.0	0.189	1.05	42.2	0.424
Na_2SO_4 average		0.25	12.0	0.209	0.85	37.9	0.415
NaCl	0.213	0.84	37.3	0.100	1.58	67.5	0.292
—	—	1.20	46,8	0.077	1.67	65.0	
—	—	0.40	16.6	0.121	1.77	74.4	
—	—	0.66	32.9	0.136	1.04	51.7	
—	—	1.20	59.1	0.139	1.67	83.2	0.288
—	—	1.37	69.4	0.093	1.25	63.5	0.292
NaCf average		0.95	43.7	0.111	1.50	67.5	0.291

observe a sodium absorption against a concentration gradient, while this only happens in a few of the ileum experiments.

Discussion.

The observed relative absorption rate of different chloride salts is previously found by HØBER (1945) and that of different sodium salts by WALLACE and CUSHNY (1899) and by HØBER (1945) in the small intestine of dogs. When comparing the series of the chloride as well as the sodium salts in the colon with that of the ileum only small and statistically insignificant differences are seen.

The experiments have further shown that the sodium ion in the colon may be replaced by a series of other cations without influencing the absorption of chloride against a concentration gradient — only the rate may be influenced.

In the ileum an absorption of chloride against a concentration gradient was not seen as the chloride concentration of the infused solutions was too high.

As to the sodium ion an absorption against a concentration gradient was observed in the colon when the chloride ion was replaced by other anions. The same phenomenon was observed in some of the ileum experiments. The sodium absorption rate was somewhat influenced by the different anions.

These experiments do not exclude that the sodium ion is of special importance to the chloride ion absorption (USSING (1949)) or vice versa, because during absorption experiments in the intestine we have always sodium as well as chloride ions in the intestinal content.

Summary.

Previously it was found that the chloride as well as the sodium ions could be absorbed against a concentration gradient in the colon and the distal part of the small intestine of rats when pure sodium chloride solutions were infused. In this paper it is demonstrated that the chloride ion is still absorbed against a concentration gradient in the colon when sodium is replaced by potassium, calcium, magnesium or ammonium, and that sodium, too, is absorbed against a concentration gradient in the colon as well as the distal part of the small intestine when the chloride ion is replaced by acetate, nitrate, bicarbonate or sulphate ions.

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From the Department of Physiology, University of Gothenburg, Gothenburg, Sweden.

The Distensibility of the Systemic Resistance Blood Vessels.

By

BJÖRN FOLKOW and BIRGER LÖFVING.

Received 1 August 1956.

At first sight experimental data pertaining to the distensibility of the resistance vessels of the general circulation present a rather confusing picture. Firstly, there are a number of well-controlled experiments indicating that the vessels behave as if they were practically indistensible (see *e. g.* WHITTAKER and WINTON 1938, PAPPENHEIMER and MAES 1942, BURTON 1952, 1954). Secondly, others present equally good evidence that they are quite distensible (GREEN, LEWIS, NICKERSON and HELLER, 1944, PHILLIPS, BRIND and LEVY 1955). Lastly, there are other data suggesting that smooth muscle reactions, counteracting distensibility, can be strong enough to induce a net decrease of the vascular lumina in some regions when pressure is raised (BAYLISS 1902, FOG 1934, 1937, 1938, FORBES, NASON and WORTMAN 1937, FOLKOW 1949, 1952 and others). These apparently contradictory results are easily explained if it is assumed that the resistance vessels are distensible but this 'passive' change of their lumina is normally more or less counteracted by 'active' changes of vascular tone (FOLKOW 1952a, 1956a). As this type of smooth muscle reactions are easily influenced by even minor interferences with the tissues or the blood, they are very variable in extent and often almost abolished if vascular tone is depressed (FOLKOW 1952b). Further, there seem to be considerable differences in these reactions between different vascular areas. Keeping this in mind it is obvious why the pressure-flow curves, from which changes in vascular

radius with changes of blood pressure are generally deduced, can be so highly variable. See also the review of WEZLER and SINN (1953.)

In the present study attempts have been made to estimate the distensibility of the systemic resistance vessels at different, known levels of vasoconstriction under circumstances in which adaptive changes of smooth muscle activity were practically eliminated. — A brief, preliminary report of this study has previously been given (FOLKOW 1953).

Methods.

Experiments were performed on 26 cats, under chloralose anaesthesia, utilizing predominately the vascular region of the acutely denervated lower part of the right hind limb. The abdominal sympathetic trunks were extirpated and the suprarenal glands denervated in order to eliminate all possible sympathetic influences on the tone of the limb vessels. The limb was prepared in such a way that it was connected to the animal only by the main blood vessels at the knee joint level. All side-branches from the level of the aorta and the inferior caval vein down to the knee joint were ligated. The paw circulation was eliminated by a tight ligature at the ankle joint, so that the preparation included only the vascular bed of the calf, perfused by way of its cognate artery and vein.

This vascular region was first perfused with blood in the normal way from the animal itself. The blood flow was generally recorded from the isolated femoral vein by an optical drop recorder, operating an ordinate writer. The ordinates were approximately inversely proportional to the rate of flow. The unit was also adapted for recording the arterial inflow (for principles see LINDGREN and UVNÄS 1954). This was in some experiments measured also by a sensitive differential manometer (PAPPENHEIMER 1954), connected to a Sanborn twin-viso recorder, which recorded the variations in pressure drop across a short narrow tube, connecting the abdominal aorta with the iliac artery. As a control the flow was now and then measured simply by collecting the venous outflow in a graded tube.

The 'arterial inflow pressure' (P_A) was measured from the left femoral artery, all other branches of the lower part of the aorta being ligated. Concomitantly the 'venous outflow pressure' (P_V) was recorded from the femoral vein. Sometimes the differential manometer was utilized for measuring the pressure drop over the perfused vascular bed. Great care was always taken to exclude the pressure drop across the flow recorders from the measured pressure drop over the vascular tree.

The isolated vascular bed was then utilized for a perfusion with oxygenated dextrane Tyrode solution at 38° C from a reservoir, connected to the abdominal aorta. The preparations were only used as long as no signs of oedema were present. Since this solution contains no cells the effects of the anomalous viscosity of blood was avoided. 'Vascular tone' (taken throughout to denote the effect of smooth muscle

activity on the resistance to flow) in such an artificial perfusion is much reduced but not completely abolished. Maximal dilatation could be induced by adding chloral hydrate to the perfusate. Poisoning the vessels with cyanide in control experiments did then not further increase the flow, which would be the case if some smooth muscle activity were still present. A graded, artificially induced vascular tone, which was extremely stable, could be established by adding barium chloride in sufficient amounts to the perfusate. The vascular smooth muscle cells seemed to be completely dominated by these gross changes in chemical environment. Thus reactive hyperaemia after a period of complete obstruction of flow was generally quite absent, indicating that vascular reactivity to physiological influences was almost completely abolished. This is necessary to stress, as variations of smooth muscle activity on changes of pressure would in fact constitute a 'shift in baseline', which would make it impossible to reveal the distending effect of the pressure. The extent of the established vascular tone could be quantitatively defined by relating the created resistance to flow to the resistance that remains at maximal dilatation. The resistance values, used for this comparison, were always measured when P_A was 100 mm Hg and P_V zero and expressed as 'peripheral resistance units', PRU (GREEN et al. 1944).

Changes in P_A were induced by a partial occlusion of the abdominal aorta, while P_V could be varied within wide limits by raising the level of the outflow drop recorder to a known height above the limb. Therefore, both the pressure drop, P_D ($= P_A - P_V$), and the 'mean intravascular pressure', P_{MI} ($= \frac{P_A + P_V}{2}$) could be varied independent of each other. P_{MI} represents the effective distending force and is by definition the pressure at a point, which divides the resistance vessels into two equally big 'arterial and venous sections'. — For known levels of vascular tone the relative changes in radius of the resistance vessels, induced by pressure changes, could then be deduced from the measured flow values and P_D and P_{MI}, utilizing the Poiseulle formula. It was then assumed that variations in the length of the vessels is negligible.

Results.

The essential results, obtained while the limb vessels were perfused by the animal itself, are illustrated in fig. 1, and are in agreement with previous studies (FOLKOW 1949, 1952 a, b). When the tone of the acutely denervated vessels is still good (A in fig. 1) a decrease of P_A — and consequently also of P_{MI} — results in a decrease of flow, which initially is relatively greater than the reduction of P_D, provided that an almost instantaneous reduction of P_A can be induced. Very soon, however, the flow starts to increase, in spite of a constantly lowered P_D, indicating a gradual reduction of smooth muscle tone during a minute or two (period

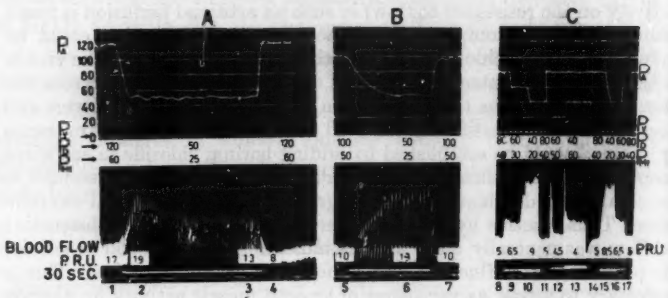

Fig. 1. Correlation between P_D, P_{MI} and blood flow:
A. While vasular tone and reactivity are still relatively good.
B. One hour later when vascular tone is reduced.
C. While the vessels are maximally dilated.
For further explanation see the text.

3 in fig. 1). This reduction of tone is revealed as a reactive hyperaemia if the initial P_D is re-established by momentarily raising P_A to its original level (period 4 in fig. 1). At the rapid shift of P_{MI} between periods 3 and 4 there is a marked decrease in resistance to flow, which in all probability is due to vascular distension, as vascular smooth muscle reactions do not occur so promptly. It is thus often possible to unmask a distensibility of the vascular walls also when counteracting smooth muscle reactions ultimately cause a net decrease of resistance when pressure is lowered. The extent of this distensibility is, however, difficult to evaluate under such circumstances.

Chart B in fig. 1 illustrates the state one hour later, when the tone of the vascular smooth muscle cells is decreased and they show little evidence of reactions on changes of pressure, as now there is no sign of reactive hyperaemia (period 7 in fig. 1). Though not yet maximally dilated the vessels behave as passive-elastic tubes, as the resistance increases when P_{MI} is lowered (compare periods 5 and 6). Their distensibility cannot, however, be deduced with any exactness, as the resistance is probably also influenced by concomitant changes in effective viscosity.

In chart C the limb vessels are brought to a state of maximal dilatation by continuous intra-arterial infusion of a concentrated acetylcholine solution. Reduction of both P_D and P_{MI} leads to a marked increase of the resistance to blood flow, while the same reduction of P_D, if combined with a raised P_{MI}, reduces the resistance (compare periods 8, 9 and 10 with 11, 12 and 13). When P_D

is kept constant the resistance varies inversely with changes of P_{MI} (compare periods 10 and 13). Such a relationship can not be ascribed to alterations in effective viscosity, but means a distensibility of the maximally dilated resistance vessels.

Attempts were also made to measure the 'critical closing pressure' (CCP), while the reactivity of the vessels was still good. It was then regularly found that on occlusion of the arterial supply the decreasing P_A did not level out at a pressure value, specific for the prevailing vascular tone. On the contrary, independent of the initial tone level, P_A generally fell continuously though slowly down to a few mm Hg above the zero level. This is not surprising, since the concomitant reduction of flow must decrease vascular tone steadily as local oxygen tension then is lowered and the metabolite concentration increases. That such a steady decrease of vascular tone occurs is revealed by the existence of the well-known reactive hyperaemia phenomenon, which is normally seen after even brief occlusions of the arterial supply.

The interference of effective viscosity variations and adaptive changes in smooth muscle tone were then eliminated by using the artificial perfusion technique. At maximal dilatation the vessels behaved like passive, definitely distensible tubes, which was evident from the decrease of resistance with increases of P_{MI} (compare periods 3, 4 and 5 in fig. 2). When, however, P_{MI} was raised above 50—60 mm Hg, vascular distensibility decreased rapidly, as then the resistance was only little affected by further increases of P_{MI} (compare periods 5 and 7).

The right hand chart in fig. 2 illustrates the extent of vascular distensibility when a component of 'active' smooth muscle tension is added to the wall elements. In this case barium chloride increased the resistance to flow to five times that at maximal dilatation. The vessels were also now quite distensible, as was evident from the changes of resistance at repeated shifts of P_{MI} (periods 10 to 14 in fig. 2).

Another experiment on maximally dilated vessels is illustrated in fig. 3. First P_A was increased stepwise at a constant P_V which resulted in a stepwise, collateral increase of P_{MI}. The pressure-flow relationship so obtained formed a curve, convex to the pressure axis, indicating vascular distension (continuous curve in fig. 3 A). — Then both P_A and P_V were varied in such a way that their difference changed but their sum remained constant. Thus P_D was varied while P_{MI} was kept constant. The pressure-flow curve

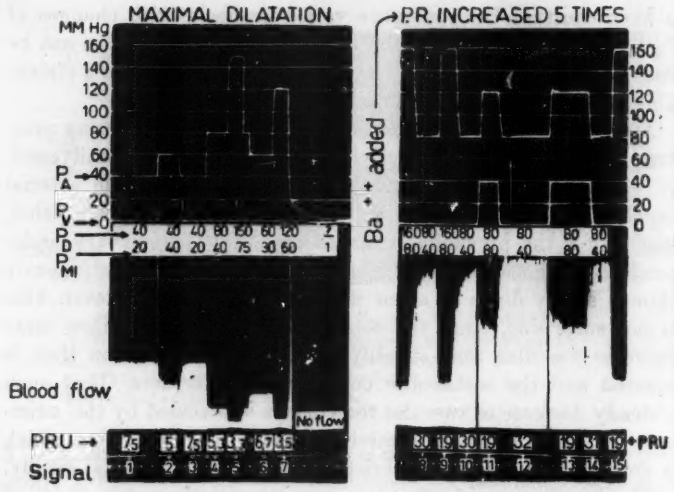

Fig. 2. Correlation between P_D P_{MI} and flow.
Perfusion with dextrane-Tyrode solutation:
A. At maximal dilatation.
B. At a steady vascular tone, induced by barium chloride.
For further explanation see the text.

now formed a practically straight line (dotted curve in fig. 3 A), which is to be expected if the distending force remained constant. In fact, this curve is slightly concave towards the pressure axis, which is to be expected, if the vessels are distensible, for the following reasons. The more thin-walled 'venous section' of the resistance vessels is probably more distensible than the 'arterial section'. The resistance to flow over the vascular bed must then be slightly lower when P_{MI} was kept constant by raising both P_A and P_V than by raising only P_A. This, because in the first alternative a bigger proportion of the distending force is put on the more distensible 'venous section' of the resistance vessels.

Then P_D was kept constant, while P_{MI} was increased by equal rises of P_A and P_V (chart B in fig. 3). This procedure decreases the resistance to flow considerably up to a P_{MI} level about 50—60 mm Hg, after which the percentage decrease of resistance with increases of P_{MI} is much reduced. It seems as if then a rigid element in the vascular wall prevents further distension.

All carefully checked data from the perfusion experiments were then utilized for the construction of the diagram in fig. 4, intended

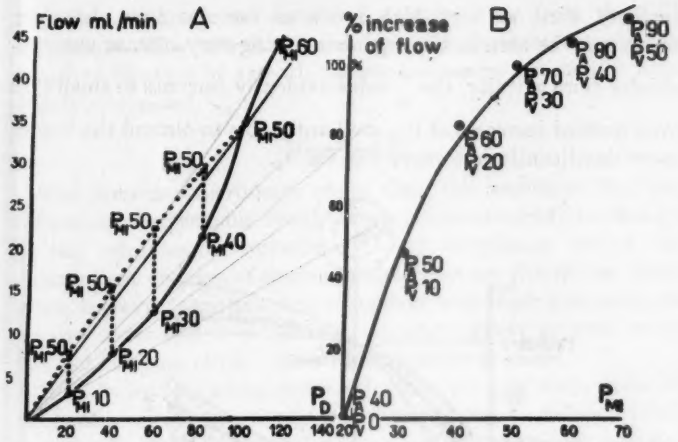

Fig. 3. Perfusion with dextrane-Tyrode solution. Limb vessels maximally dilated.
A. Continuous curve: Pressure flow relationship when both P_D and P_{MI} are increased stepwise.
Dotted curve: Pressure-flow relationship when P_D is increased stepwise at a constant P_{MI}.
B. Effect on flow when P_{MI} is increased at a constant P_D.
For further explanation see the text.

to illustrate the unhampered distensibility of the systemic resistance vessels at maximal dilatation and at different levels of tone. Under conditions in which P_A is set at 100 mm Hg, P_V at zero and the vessels are maximally dilated, flow is defined as F and the internal radius and the wall thickness of an imaginary 'unit vessel' are defined as r and w respectively. Assuming that the vascular wall mass remains the same independent of variations of tone, changes in $\frac{r}{w}$ can be deduced for each value of r, which is used here as an index of vascular tone. The effects of changes of P_{MI} on r at different levels of tone are then calculated from the pressure and flow values. Noteworthy is the marked distensibility of the maximally dilated vessels below a P_{MI} of about 50—60 mm Hg, in contrast to their relatively constant radius above this level. Curves 2—4 in fig. 4 correspond well to what can be called the normal range of vascular tone. To judge from the decreased percentage change of r at a given change of P_{MI} the vessels become less distensible at increased vascular tone, probably because then the $\frac{r}{w}$ value decreases (*i. e.* the wall gets thicker and the lumen

smaller). First at very high levels of vascular tone, however, which may be seen in the organism during very intense vasoconstrictor fibre activity, the $\frac{r}{w}$ value evidently becomes so small that even marked increases of P_{MI} are hardly able to distend the vessels more significantly (see curve 5 in fig. 4).

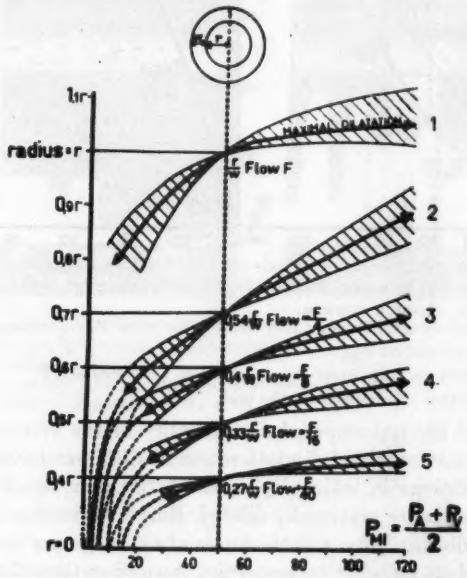

Fig. 4. The correlation between P_{MI}, representing the distending force, and r, the internal radius of an imaginary unit vessel.
Curve 1: The blood vessels maximally dilated.
Curves 2—5: At increasing, defined levels of a steady vascular tone.
The spreading between the different observations is indicated by the traced surfaces. For further explanation see the text.

At the very low ranges of P_D it is difficult to measure the pressure and the flow with great accuracy in a preparation of this type. Hence, changes in r below a P_{MI} of 10—15 mm Hg have not been calculated in detail. Instead of this, P_A at zero flow, *i. e.* CCP (when r is assumed to be zero), has been measured at each tone level by occlusion of the arterial inflow. Note the wide spread and consequent overlap of the CCP values for different tone levels, which in the individual case made it difficult to predict

with any accuracy the extent of vascular tone from the CCP value only. This in spite of the fact that in these experiments adaptive changes of smooth muscle tone were practically completely eliminated.

Discussion.

The present experiments show that the resistance to flow, offered by the systemic blood vessels, varies inversely to changes of the intravascular pressure, if only interfering factors like concomitant changes of smooth muscle tone are eliminated. Quite often, however, counteracting changes of tone occur and mask the passive distension to simulate a vascular rigidity or even result in a net decrease of the lumen when pressure is raised.

BURTON and his group assume that the vascular walls under all circumstances are practically indistensible, due to collagen fibrils that are thought to be anchored to and automatically adjusted by the distensible smooth muscle cells. It is further postulated that below a 'critical' pressure level, characteristic for each level of vascular tone, the vessels suddenly must close completely (see BURTON 1954). — BURTON suggests a morphological arrangement of muscle and fibrous elements that results in perhaps as much as a ten times increased force, acting on the collagen fibrils to keep them tightened, by allowing the smooth muscle cells to shorten ten times more. However, the smooth muscle cells of the resistance vessels have a practically circular arrangement, which the collagen fibrils essentially must also have if they are to be able to withstand radial distension. If now the smooth muscle cells shorten ten times more than the connected collagen fibrils, it follows that the latter must become slack. Then they give no rigid support whatsoever to the wall, thus leaving the distensible smooth muscle layer to offer the main resistance to stretch (see also FOLKOW 1956 a).

It is not only of academic interest to study the extent of vascular distensibility at different levels of tone and how 'active' changes of smooth muscle tone sometimes can make the vessels behave as if they were rigid, or even decrease their lumina when pressure is increased. This, because these 'active' reactions sometimes are quite abolished and on the whole vary considerably, not only with the vascular region concerned, but also with the prevailing reactivity of the vessels. The cerebral vessels, for instance, have a pronounced 'basal tone', mainly regulated by the

local influence of oxygen tension and vasodilator metabolites, which sensitively adjust the blood flow to keep in balance with the metabolic needs (see *e. g.* FOLKOW 1955). Here vascular distensibility is quite overshadowed by counteracting changes of tone, as is evident from the observation that cerebral vascular resistance is generally lowered when blood pressure falls (see *e. g.* SCHMIDT 1950, FINNERTY, WITKIN and FAZEKAS 1954, MOYER and MORRIS 1954). — Such adjustments of vascular tone are to a variable extent seen in most vascular areas. They seem to be partly due to the inevitable variations in the local concentrations of vasodilator metabolites and oxygen, that must take place when pressure and blood flow are varied at a constant tissue metabolism. Further, the distending pressure as such can evidently under some circumstances act as a stretch stimulus on the smooth muscle cells of the blood vessels, thus contributing to the external excitatory influences that steadily moderate their activity (BAYLISS 1902, FOLKOW 1949, 1952a, HILTON 1953 and WOOD, LITTER and WILKINS 1955). The less vitally important a tissue is, however, the more are centrally directed vasomotor reflexes allowed to dominate over these local adjustments of vascular tone (see FOLKOW 1955). Therefore, to reveal their existence, *e. g.* within the skeletal muscle area, it is generally necessary first to eliminate all nervous influences. In still other areas, *e. g.* in the skin with its arterio-venous anastomoses, smooth muscle adaptations to changes in pressure and flow seem to be quite small, as here a decrease of resistance is regularly seen on increase of blood pressure (see GREEN et al. 1944, FOLKOW 1952a).

All blood vessels will, however, behave more and more like passive-elastic tubes when their tone is reduced, as then compensatory smooth muscle reactions are mostly depressed (compare A, B and C in fig. 1). Thus, even for one and the same vascular region it can never be expected to exist a standard pressure-flow curve, characteristic for that very vascular bed. This is important to realize as is illustrated by the following examples. Suppose that anoxaemia or a partial obstruction of a main artery has induced an almost complete, compensatory dilatation of the cerebral vessels. A passive-elastic recoil now dominates the response of these vessels when pressure is reduced, decreasing flow proportionally more than the pressure. Even a moderate fall in blood pressure can then in a dangerous way interfere with the nutrition of the brain, while such a pressure fall under normal circumstances

would very little decrease the cerebral blood flow. — To illustrate further the importance of analyzing the basic haemodynamic events associated with pressure changes, the situation in chronic hypertensive disease can be mentioned. Here hypertrophic and later on sclerotic vascular changes generally take place, which probably result in a decrease of vascular distensibility, not to mention other haemodynamic problems that may arise (for details see FOLKOW 1956b). A fundamental shift in physical equilibrium of the vessels may occur and they cannot simply be compared with the behaviour of a normal vascular bed at increased tone and acutely exposed to a raised pressure.

With regard to the concept of the 'critical closing pressure' (CCP) BURTON and his group (see BURTON 1951, 1952, 1954) assume that for each tone level there would exist a 'critical' pressure when even a slight increase of the tension of the indistensible vascular wall induces a sudden occlusion of the lumen. At pressures well above the 'critical' level, on the other hand, increased smooth muscle tone should result in a graded lumen decrease. — A complete closure of a vessel would be natural if it formed the end result of a gradual elastic recoil of distensible vascular walls. Provided occlusion is not caused by a tissue pressure, higher than the intravascular pressure, or a sudden 'active' increase av smooth muscle tone, it is difficult to see, however, what force should induce a 'critical closing' of indistensible vessels. One then has to imagine that an increase of wall tension *remained* increased in spite of the resulting shortening of the wall. Only under such circumstances it would be possible to enforce a continuous constriction down to a complete closure. It then follows, however, that, independent of 'critical' pressure levels, any increase of vascular smooth muscle tone should result in vascular occlusion and this is certainly not the case. On the contrary, a given increase of vascular smooth muscle tension must be 'consumed' when it is transformed into a shortening. After a constriction, proportional to the initial increase of wall tension, a new equilibrium must be reached between wall tension and distending pressure. A 'critical' vascular closing of the type BURTON suggests is, in our view, impossible as long as it is assumed that the vessels are essentially indistensible.

No doubt, however, a type of reaction that deserves to be called a 'critical closing' can occur, if only concomitant decreases of smooth muscle activity are eliminated (see fig. 4). Even if the

vessels prove to be distensible at different levels of tone, the radius decrease per unit decrease of P_{MI} is not pronounced enough to result gradually in a complete occlusion. The closing is therefore not a simple consequence of a gradual elastic recoil. On the contrary, the decrease of the internal radius for the 'unit vessel', representing the vascular bed as a whole, is quite suddenly accelerated at lower P_{MI} values to a complete obstruction. This characteristic of the curves in fig. 4 is best explained as due to a marked elastic recoil of only a limited, highly distensible section of the parallel tubings, while at higher pressure levels the other sections of the vascular tree dominate the slope of the curves with their more moderate distensibility.

It is since long known that a limited section of the vascular tree, representated mainly by the praecapillary sphincter regions, normally shows unsynchronized, rhythmic contractions, strong enough to close the lumen completely. For this reason flow in the majority of the capillaries in resting skeletal muscles is obstructed at any given moment. If all these sphincter areas under specific circumstances are simultaneously activated it should result in a complete cessation of flow. To analyze the background of such a reaction it is necessary to discuss the physical characteristics of the wall elements. Both intimal and smooth muscle tissue are probably highly distensible, possibly much more so than the elastic membranes, present only in somewhat bigger vessels. Around these layers there seems to be an essentially indistensible collagen network, which sets the outer limit for the lumen (see BURTON 1954). The sphincter section of the vascular tree seems to have a relatively thin intimal and muscle tissue, which must mean a high distensibility, where only the fibrous jacket sets an outer limit for further distension. Such a construction is favourable for the occurrence of an elastic recoil from the wide-open state down to a complete obstruction over a narrow, 'critical' range of pressure. Such a closure does not necessarily mean a more remarkable shortening of the sphincters. If in the wide-open state the lumen of such a vessel is 10 microns, its intima 1 micron and the muscle sphincter 2 microns thick, a wall layer of 2 microns is situated within the main line for smooth muscle shortening. Only a 30 per cent shortening of the sphincter cell is then needed for a closure of the lumen. The probably increased elasticity modulus when the sphincter is activated would raise the 'critical' pressure range, but still keep the needed increase of pressure for

complete opening up the vessel relatively small. From a practical point of view such a vessel would either be maximally open or completely closed with only a narrow pressure range left for intermediate states. — The relative influence of this vascular section on the total resistance is probably very much bigger at the lower range of P_{MI}. If at normal pressure say one fifth of the sphincters is open and then contribute with for instance 20 per cent of the total resistance, this resistance would only decrease by about 15 per cent if the remaining four fifths of the channels were forced open by a given pressure increase. If, on the other hand, from the same initial state an equal fraction of them were closed by a similar decrease of pressure, leaving one twenty-fifth of the channels open to flow, the total resistance would increase about 80 per cent. At further closure of additional sphincters on lowering of P_{MI} their influence on the total resistance would increase more and more rapidly, until none of them remains open, when the 'critical closing pressure' would be reached. The sudden decrease of r in fig. 4 at low P_{MI} values does therefore not necessitate that all sphincters suddenly close almost simultaneously.

In specialized vessels dominated by a thick muscle layer, like arteriovenous anastomoses, distensibility of the wall must be somewhat reduced but on the other hand a less pronounced muscle recoil is then needed for a lumen occlusion.

With regard to the other sections of the vascular tree, arterioles etc., it may be questioned whether they are significantly distensible. They would then be mainly responsible for the more even radius increase when P_{MI} is increased above the 'critical' range (see fig. 4). It might be argued that also this resistance decrease is purely a matter of additional opening up of previously closed precapillary vessels, increasing the number of parallel channels. The consequences as to the total resistance would be the same in the two alternatives, though the latter also implies an increase of the functioning capillary exchange surface. No doubt the vascular tree as a whole is quite distensible at the maximally dilated state where all smooth muscles should be relaxed and therefore all parallel channels open (curve 1 in fig. 4). A moderate smooth muscle contraction means that the muscle layer must partly unload the other wall components with no longer any support of the collagen fibrils even at P_{MI} values above 50—60 mm Hg. This muscle layer, which from studies of larger arteries is known to be quite distensible (BURTON 1954), must now largely be re-

sponsible for the resistance to stretch. The shift in $\frac{r}{w}$ ratio is not so big between curves 1 and 2 in fig. 4, that this factor alone should make distension impossible. For these reasons the change of r with changes in P_{MI} in curves 2—5 is probably to a great extent due to a definite distension of already open vessels, like smaller arteries, arterioles, venules etc. To judge from the slopes of the curves, however, this distensibility must be moderate. When smooth muscle tone is present, the pressure will only exceptionally be able to stretch these vessels out to the limit set by the collagen fibrils, as curves 2—5 never reach curve 1 within reasonable P_{MI} levels. The moderate distensibility of the vascular tree as a whole makes it less likely that a 'critical closing' of the type suggested for the praecapillary sections occurs for other parts of the resistance vessels. Fig. 3 in the paper of NICHOL, GIRLING, JERRARD, CLAXTON and BURTON (1951) also gives the impression that such a closing is predominately a matter of the smallest praecapillary vessels.

Also from a more practical point of view our experience with the 'critical closing pressure' differs from that of BURTON and his group. We could not find that recording of CCP in a normal vascular region with a good reactivity of the vessels could be used as a reliable indicator of prevailing vascular tone. The reason for this is that on lowering the pressure, and thus the blood flow, a concomitant decrease of vascular tone practically always takes place, so that P_A falls slowly down to quite near the zero level, whether the tone was initially marked or negligible. This is in agreement with the recent findings of LEVY, PHILLIPS and BRIND (1954).

Summary.

The distensibility of the systemic resistance blood vessels has been quantitatively measured at known levels of a steady vascular tone. Their distensibility is, from a haemodynamic point of view, considerable except at more intense vasoconstriction, where the lumen-to-wall ratio is decreased enough to minimize the distending effect of the intravascular pressure.

Often, however, vascular distension is more or less balanced by adaptive changes of smooth muscle tone. Therefore, a standard pressure-flow relationship cannot be given, as this relationship

varies, not only with the region studied, but also with the level of vascular tone and reactivity.

Generally, a 'critical closing pressure', correlated with the prevailing tone, can only be recorded if the normally occurring, adaptive changes of smooth muscle tone are eliminated. The basis for this closing is discussed at length. Reasons are presented that it might simply be an extension of the well-known intermittent closings of the praecapillary sphincter regions to include all parallel channels at this level. It is suggested that the complete closing of this section of the vascular tree is a consequence of a pronounced distensibility of the thin intima and muscle layers of these small vessels, where an outer limit for distension is set by a surrounding network of collagen fibrils.

Acknowledgement.

The expenses of this investigation were partly defrayed by a grant from the Swedish Medical Research Council. — The authors are indebted to AB Pharmacia, Uppsala, for a generous supply of dextrane.

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Institute of Physiology, University of Lund, Sweden.

Functional Organization of the Dorsal Spino-Cerebellar Tract in the Cat.

IV. Synaptic Connections of Afferents from Golgi Tendon Organs and Muscle Spindles.

By

A. LUNDBERG and O. OSCARSSON.

Received 2 August 1956.

In previous experiments with single fibre recording from the Flechsig's fasciculus, neurons were found which could be activated either by group I muscle afferents alone or by both group I and II afferents. These fibres were assumed to constitute the dorsal spino-cerebellar tract (DSCT) (LAPORTE, LUNDBERG and OSCARSSON 1956 b). Evidence for excitatory action to these neurons from muscle spindles was found in experiments with adequate activation of the muscle stretch receptors (LAPORTE and LUNDBERG 1956).

The present work is concerned with synaptic connections of Golgi tendon organ afferents as well. BRADLEY and ECCLES (1953) have shown that the group I spike from thigh muscle nerves may be double with a marked difference of thresholds and concluded that the two components are group Ia and Ib, being respectively the fibres from muscle spindles and Golgi tendon organs.

This subdivision of the group I spike has been made use of for exploration of the segmental connections of muscle spindle afferents and Golgi tendon organ afferents (BRADLEY and ECCLES 1953, ECCLES, FATT, LANDGREN and WINSBURY 1954, ECCLES, FATT and LANDGREN 1956) and in the present investigation the synaptic connections of these two group I components to DSCT neurons have been investigated. In the following the term Ia and Ib volley will be used descriptively to denote the first and

the second component in the group I spike irrespective of the fact that it is not known whether the two groups of fibres correspond to muscle spindle afferents and Golgi tendon organ afferents to the completely satisfactorily degree that is needed for determining the synaptic connections of these two afferent systems to DSCT neurons.

It will be shown in the first part of this paper that some of the DSCT neurons are discharged by a group I volley only if it includes part of Ib, but that many of the neurons receive excitatory action from both components of group I. The second part is concerned with adequate activation of stretch receptors. It will be demonstrated that some DSCT neurons are activated from Golgi tendon organs. For methods see previous papers (LAPORTE, LUNDBERG and OSCARSSON 1956 a, b, LAPORTE and LUNDBERG 1956).

Results.

1. Contribution from the Ia and Ib volleys to the discharge in DSCT neurons.

On triphasic recording from the root entry zone we could on stimulation of the quadriceps or biceps p. + semitendinosus nerves confirm the occurrence of two components of the group I spike described by BRADLEY and ECCLES (1953). To some degree this separation was noted in almost all experiments and it appeared to be complete in about every third cat.

LLOYD and MCINTYRE (1950) found that the group I volley could be traced for some distance proximally in the dorsal column and that the conduction velocity decreased with the expected relay at the caudal end of Clarke's column, a few segments above the root entry zone.

The first problem to consider is whether the Ib fibres reach this region. That this is the case is shown in Fig. 1. Recordings were made from the L VII dorsal root entry zone (records 1 and 4) and from the dorsal column 25 and 40 mm more cranially (records 2 and 5 respectively 3 and 6). Two different stimulation strengths were used. The upper records show maximal group Ia volleys and the lower records maximal group Ia + Ib volleys. The magnitude of the recorded potentials decreases as the electrode is moved cranially more or less in parallel for the two volleys. The conduction velocity of the fastest fibres in the group

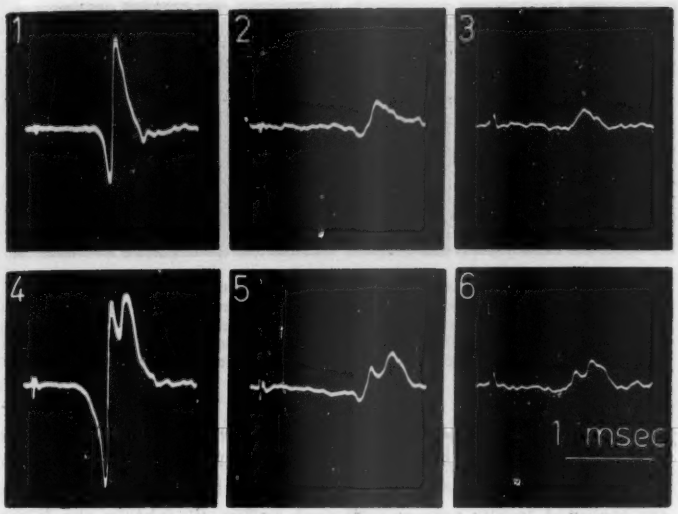

Fig. 1. Recording of Ia and Ib volleys from the dorsal column. Stimulation of biceps p. + semitendinosus nerves. In records 1—3 the stimulus strength was adjusted to give a maximal group Ia volley, in records 4—6 to give maximal Ia + Ib volleys. Triphasic recording from the L VII dorsal root entry zone (records 1 and 4) from the dorsal column 25 mm (records 2 and 5) and 40 mm (records 3 and 6) more cranially. In records 3 and 6 the stimulation preceded the start of the sweep (artifact shown 0.6 msec after stimulus).

Ia volley is initially 101 m/sec to decrease to 62 and 28 m/sec respectively at the two cranial recording places.

Fig. 2. illustrates an experiment made to decide how Ia and Ib contribute to the discharge in the dissected Flechsig's fasciculus. The primary afferent volley was judged by triphasic recording from the dorsal root entry zone in order to judge the separation in Ia and Ib and also from a dorsal root filament to see when the group II fibres were involved. Record 3 shows the maximal group Ia volley with a small addition of Ib. When the primary afferent volley includes more Ib (records 4—6), the size of the transmitted discharge recorded in the dissected fasciculus increases. The number of group II fibres activated in record 6 is too small to bring about the second spike and the late discharge which these fibres contribute to the discharge in the dissected fasciculus (LAPORTE et. al. 1956 a).

The contribution by Ib to the transmitted discharge varies considerably from experiment to experiment. Three examples are

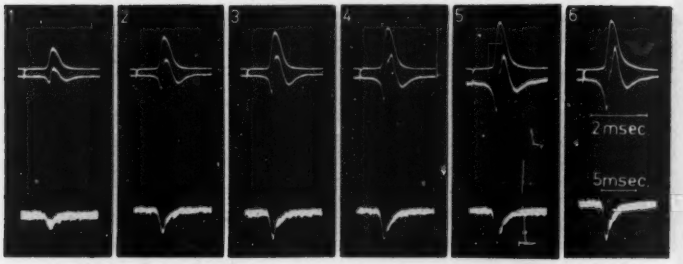

Fig. 2. Contribution by Ia and Ib to the transmitted discharge in the DSCT. Stimulation of the quadriceps nerve. Simultaneous recording in order from above downwards: from L V dorsal root filament, triphasically from L V dorsal root entry zone and from the dissected Flechsig's fasciculus. About 50 superimposed sweeps were photographed to obtain each record.

given in Fig. 3. The curve in the middle illustrates an experiment in which the addition to the discharge resulting from Ib is very small whereas in the lower curve the size of the transmitted volley is considerably increased when all the Ib fibres are included in the primary afferent volley. To obtain the lower curve the biceps p. + semitendinosus nerve was stimulated and the postsynaptic contribution from the group Ib volley was in that case often larger than when stimulating the quadriceps nerve, but the increase in the present experiment is exceptionally large. It should further be noted that the two upper curves are discontinuous and suggestive of contribution from two systems.

The next step was to find out how these two primary components of group I fibres contribute to the discharge in the single neuron when recording intra-axonally as described by LAPORTE et al. (1956 b). Most of the neurons are discharged by a Ia volley, but it was found that Ib may contribute in different ways. Some neurons were found in which a monosynaptically transmitted spike appeared only with Ib and usually even with a small Ib volley. The records in Fig. 4 are also suggestive of excitatory action by Ib. Records 1—4 show how with increasing strength of stimulation the group Ib volley grows. Only when the group Ib volley is about 2/3 maximal is there a discharge in the secondary neuron. With recording from the filament it can be observed that with respect to threshold the separation Ib and II is quite good. With the stimulus strength needed to obtain record 3 there is very little or no group II component in the filament electrogram. The latency of the spike is somewhat less than that

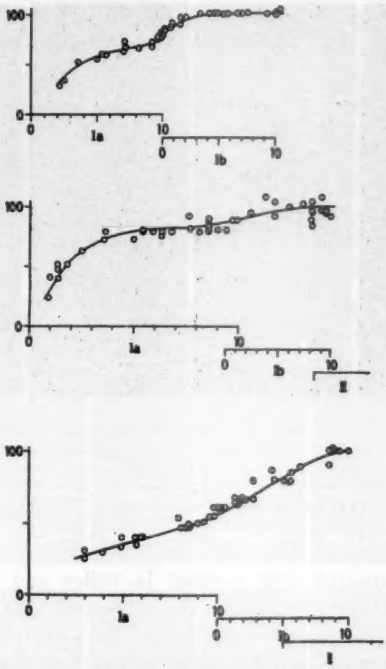

Fig. 3. Contribution of Ia and Ib to the discharge in the DSCT in three different experiments. In the two upper curves stimulation of the quadriceps nerve in the lower of biceps p. + semitendinosus nerves. In each curve the ordinate represents the height of the volley in the dissected Flechsig's fasciculus in per cent of the volley obtained on maximal stimulation of group I afferents, and the abscissa height of primary afferent Ia and Ib volley.

of the early part of the discharge in the dissected fasciculus recorded 70 mm more cranially, and with this brief latency the excitatory action cannot have been carried by group II fibres.

In many of the DSCT neurons a second spike appears only when group II fibres are included in the primary afferent volley, but sometimes a double spike may appear with a stimulus that is subthreshold to group II (Laporte et al. 1956 b). Also in the present experiment we have seen this double spike response with stimulation of group I fibres. We have in such cases tried to determine whether the two components of the group I spike both contributed to the postsynaptic discharge in the single neuron, but have failed to obtain clearcut results. Usually the

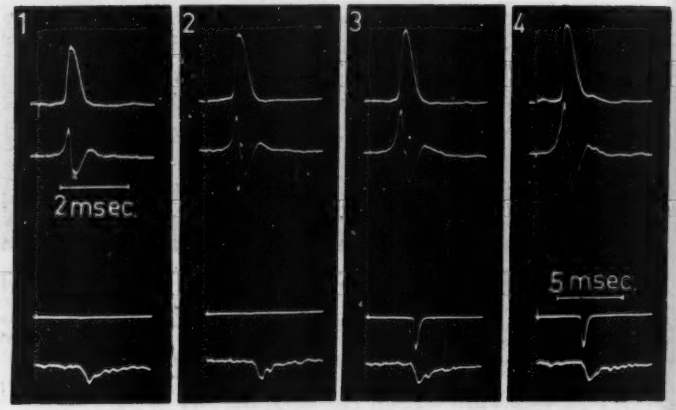

Fig. 4. Excitatory action by a Ib volley. Simultaneous recordings in order from above downwards from L V dorsal root filament, triphasically from L V dorsal root entry zone, intra-axonally from DSCT fibre and from dissected Flechsig's fasciculus. Stimulation of the quadriceps nerve. Distance dissected fasciculus — site of microelectrode recording 70 mm.

first spike appeared with a small Ia volley and the second on the border between Ia and Ib. It is possible that in some cases the first spike appeared with Ia and the second with Ib but in no case could it be entirely excluded that both the post-synaptic spikes appeared either with Ia or Ib. In none of the neurons which discharged with a single spike to a Ib volley did stimulation of group II fibres provoke a second spike.

The finding that some neurons only are activated by a group Ib volley should be expected from the fact that the mass discharge recorded in the dissected Flechsig's fasciculus increased when Ib fibres were stimulated. Some evidence indicating convergence of excitatory action by Ia and Ib to the same neuron is provided in experiments of a type illustrated in Fig. 5. As shown by records 1—6 the neuron gives a typical double spike response; the first spike appearing with low threshold group Ia fibres and the second with high threshold group II fibres in the quadriceps nerve. Seemingly Ib does not contribute to the discharge. However, to obtain record 8 the quadriceps nerve was conditioned with a volley slightly supramaximal to group Ia. A testing volley was set up by a stimulus applied in the refractory period of the conditioning volley. The testing stimulus (shown

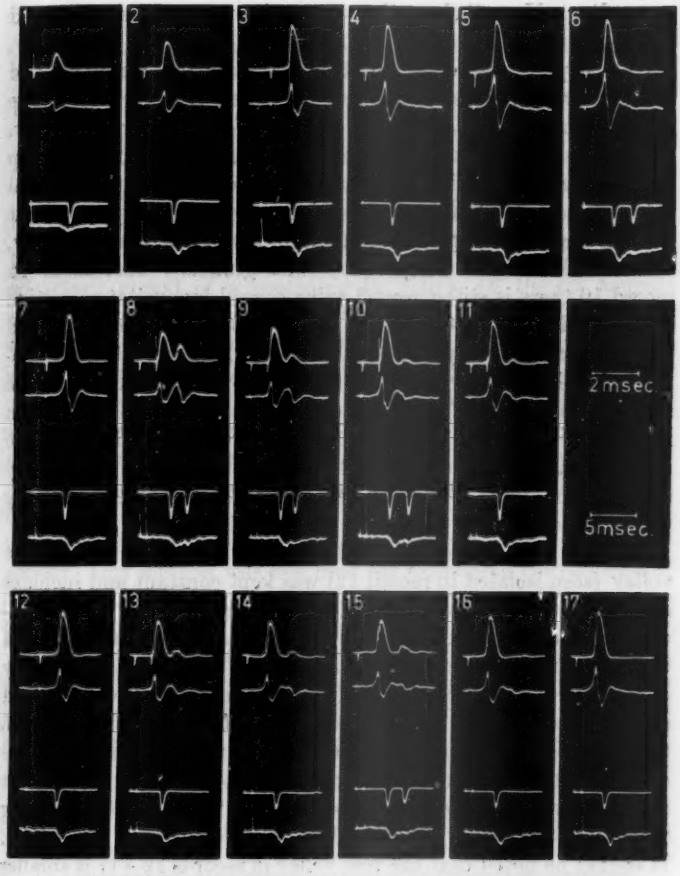

Fig. 5. Convergence of excitatory action by Ia, Ib and II. In each record stimulation of the quadriceps nerve and simultaneous recordings in order from above downwards: from L VI dorsal root filament, triphasically from L VI root entry zone (observe that positivity unconventionally is signalled by an upwards deflection), intra-axonally from DSCT fibre and from the dissected Flechsig's fasciculus. In records 1—6 increasing strength of stimulation demonstrating excitatory actions by Ia and II. In records 8—11 and 13—17 double shock stimulation demonstrating excitatory action by Ib volley. See text. Distance dissected fasciculus — site of microelectrode recording 54 mm.

in isolation in record 7) would excite approximately half Ib, but was subthreshold to group II as judged from the filament electrogram, and refractoriness would exclude all group Ia fibres from

responding. Nevertheless the test volley generates a second post-synaptic spike.

By the procedure of stimulation described (cf. BRADLEY and ECCLES 1953) the testing group Ib volley reaches the Clarke neuron about 1 msec later than the conditioning group Ia volley. This interval is necessary in order that excitatory action by group Ib may generate an additional spike. In records 9—11 the interval between the conditioning and testing volleys was kept constant as also was the strength of stimulation giving the testing volley, whereas the conditioning volley was gradually increased. In record 10 there is still a second spike caused by excitatory action by Ib in the range between Ib in the test volley (record 7) and Ib in the conditioning volley in record 10. The potential contributed in the primary afferent fibres appears directly as the second component of the filament electrogram (upper beam in record 10). In record 11 the conditioning volley is slightly more increased and the number of fibres activated by the test volley in the refractory period of an almost as large a conditioning volley is not enough to give a second spike.

Records 12—17 were obtained from the same neuron. The test volley (seen isolated in record 12) was kept constant and includes about half as much of Ib as the test volley in record 7. The conditioning volley in records 13—15 is the same as in record 9. With the same testing interval as with records 8—11 the testing volley does not give a second spike. On lengthening the interval a second spike appears (record 15). The second spike is not due to restimulation of fibres activated by the conditioning volley (as might be the case with this long interval) for both it and the later component in the primary afferent volley disappears when the conditioning volley is increased to be larger than the testing volley (record 17). It should be noted that the test volley in records 12—17 is smaller than the conditioning volley in record 10 and hence that excitatory action to this secondary neuron is supplied by fibres in two different ranges of the Ib spectrum.

Convergence of this type was found frequently. Table 1 summarizes the findings from three experiments. 28 neurons excited by group I fibres in the quadriceps nerve were analysed as illustrated in Fig. 5. When using single shock stimulation 24 of these units were excited from Ia, 2 on the verge between Ia and Ib (probably by a weak Ib volley) and 2 only when the volley included group Ib. Of the 24 neurons excited from group Ia

Table 1.

Convergence of excitatory action from one muscle to individual DSCT neurons.

Ia	Ia — Ib	Ib	Ia + Ib	Ia + II	Ia + Ib + II	Total
3	2	2	11	3	7	28

18 were excited also from Ib when activated separately in the refractory period of a preceding Ia volley. Of the 28 neurons examined only 10 gave a second spike with group II, which is somewhat less than usually observed. Subliminal excitatory action is not included in Table 1 and was sometimes found. Some secondary neurons fired on single shock stimulation only when Ib was included but discharged with a double Ia volley. Furthermore in some of the secondary neurons in which a single spike resulted on simultaneous stimulation of group I and II fibres, it was noted that if group II fibres were stimulated in the refractory period of a proceding group I volley a second spike may appear. This method was not used for examining convergence of excitatory action by group I and II fibres to any of the neurons listed in Table 1.

It was found (LAPORTE et al. 1956 b, c) that the DSCT neurons were inhibited by group I fibres from antagonist muscles. We have now examined this inhibition with respect to the contribution from the two group I components. Table 2 summarizes

Table 2.

Convergence of inhibitory action to individual DSCT neurons from the antagonist of the muscle supplying excitatory action.

only Ia	only Ib	Ia + Ib	no effect	fac.	Total
4 + (1)	6 + (1)	9 + (7)	1	2	31

the results obtained from neurons excited either from the quadriceps nerve or from the biceps p. + semitendinosus nerves. Inhibition was indicated by cessation or decrease in frequency of resting discharge by repetitive stimulation at 100/sec of the severed nerve from the antagonist muscle. Brackets denote weak effect.

Fig. 6. Records from three DSCT axons (I: records 1—4, II: records 5—6 and III: records 7—8) during isometric contraction of the soleus muscle. These neurons could be activated on electrical stimulation of group I fibres in the triceps nerve and discharged on stretch of soleus. Resting tension in all neurons about 500 g (records 1, 5 and 7). Acceleration of discharge in neuron I and III and pause in neuron II during isometric single twitch in the soleus muscle.

It is evident that inhibitory action from antagonist muscles frequently is also carried both in the Ia and Ib volleys.

The question as to whether group II contributes to the inhibition was not systematically studied, but occasionally, when inhibition was not complete with group I, it was noted that the inhibitory effect increased with stimulation supramaximal for group I but subthreshold for group III afferents, indicating that also group II fibres may contribute inhibitory action to DSCT neurons excited by group I afferents from antagonist muscles.

2. Unit discharge in DSCT neurons during contraction of muscle supplying group I excitatory action.

The results described in the first part indicate that primary afferents from Golgi tendon organs as well as afferents from the muscle spindles may have excitatory action on DSCT neurons. During previous work with the use of adequate stimulation (Laporte et al. 1956 c) it was noted that a post-synaptic discharge elicited from quadriceps paused with contraction of the muscle, thus giving evidence of excitatory action from spindles. For work on receptor properties the Golgi tendon organ discharge was examined mainly from the soleus muscle (Matthews 1933, Hunt and Kuffler 1951) which has a long contraction

time and therefore gives a longlasting discharge with a single twitch.

In the present series of experiments it was therefore natural to start out with neurons which could be excited mono-synaptically from low threshold afferents in the triceps nerve and responded to a pull of the soleus muscle. The time course of the isometric contraction was measured with a strain gauge. Very soon it became evident that in some neurons the discharge accelerated during contraction. Records 1—4 in Fig. 6 were all obtained from a "soleus neuron" which was silent at rest. On moderate stretch the discharge observed in record 1 appeared. With stimulation of the triceps nerve the discharge accelerates already with a weak contraction of the soleus muscle and the frequency of the discharge increases with increasing tension. Records 7 and 8 were obtained from another "soleus neuron" and here the frequency of the discharge is as high as 150/sec. This was the highest frequency ever found in a neuron excited from soleus. Records 5—6 finally are also from a "soleus neuron" but in this case the discharge pauses with contraction. This pause was found on isotonic as well as on isometric contraction.

Fig. 7 demonstrates similar experiments on two neurons which were mono-synaptically activated by low threshold afferents in

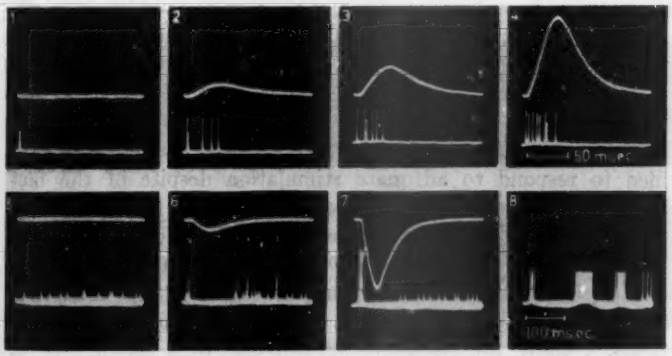

Fig. 7. Records from DSCT fibres during contraction of the gastrocnemius muscle from two neurons (I: records 1—4 and II: records 5—8) which could be activated by electrical stimulation of group I fibres in the triceps nerve and discharged on stretch of the gastrocnemius muscle. Acceleration of discharge in neuron I and pause in neuron II during isometric single twitch of the gastrocnemius muscle. Record 8 shows intermittent high frequency discharge in neuron on contraction of the gastrocnemius muscle with a weight of 500 g attached to the tendon.

the triceps nerve, but discharged when the gastrocnemius muscle was stretched. Records 1—4 are from a neuron which was silent at rest and responded to a strong pull of the muscle with a low frequency discharge subsiding with time so that the neuron was almost silent when these records were taken. In record 1 the low threshold afferents were excited to discharge the secondary neuron, but stimulation was subthreshold to the efferent fibres. With increasing strength of stimulus the tension developed by a single twitch appears in the strain gauge record and with that also a discharge in the secondary neuron during the contraction phase. Characteristically there is no discharge during relaxation. For comparison a recording was made from primary afferents connected to Golgi tendon organs in the quadriceps and gastrocnemius muscles and the acceleration of the discharge during contraction was found to last for periods comparable to those as found in Figs. 6 and 7 respectively.

Records 5—7 in Fig. 7 for comparison show a discharge, which pauses with isometric contraction of the gastrocnemius muscle. To obtain record 8 the muscle was loaded with 500 g and contracted isotonically. The intermittent high frequency discharge is characteristic for discharge in primary afferents from muscle spindles. These records are similar to those presented by LAPORTE et al. (1956 c) from experiments in which ventral roots were stimulated to provoke contraction of the muscle and characteristic of what should be expected with excitatory action from muscle spindles.

During the previous work with adequate stimulation of stretch receptors it was noted that, even with a strong pull of the muscle, the discharge in the secondary neuron had low frequency or failed to respond to adequate stimulation despite of the fact that the neuron could be excited on electrical stimulation of group I afferents from the same muscle. We have confirmed these observations. The low frequency of post-synaptic discharge is found also in neurons which judged from the acceleration of the discharge during contraction are excited from Golgi tendon organs. Usually the frequency in response to a strong pull of the tendon does not exceed 20/sec.

From the behaviour of the discharge during stretch it was often possible to predict whether a neuron would pause or accelerate during contraction. Many of the neurons with pause responded to a slight stretch of the muscle and with a rapid stretch

there was usually a burst of impulses in the secondary neuron such as occurs with primary afferents of annulo-spiral endings (MATTHEWS 1933). The secondary neurons which accelerated during contraction always needed a strong pull to respond and were not affected by a rapid stretch of the muscles as also should be expected from the known properties of the receptor. Some of the neurons which subsequently turned out to pause during contraction deviated from this scheme and did only discharge to a strong pull.

Results presented in the first part of this paper demonstrated that many DSCT neurons may be activated both by Ia and Ib volleys. Consequently it is of interest to learn if convergence of excitation from muscle spindles and Golgi tendon organs can be found with adequate activation of these stretch receptors. The plan was to identify neurons which accelerated during isometric contraction and examine if they paused on isotonic contraction. Record 1 Fig. 8 shows the longlasting discharge in the secondary neuron on stimulation of the triceps nerve. The early discharge in the primary afferent is presumably responsible for repetition (cf. LAPORTE et al. 1956 c). In records 2—4 gastrocnemius was stretched to a resting tension of about 500 g. During isometric contraction of the muscle the discharge accelerates (records 3—4). Then the tendon of the gastrocnemius muscle was strongly pulled by hand with the discharge in record 5 resulting. With continued

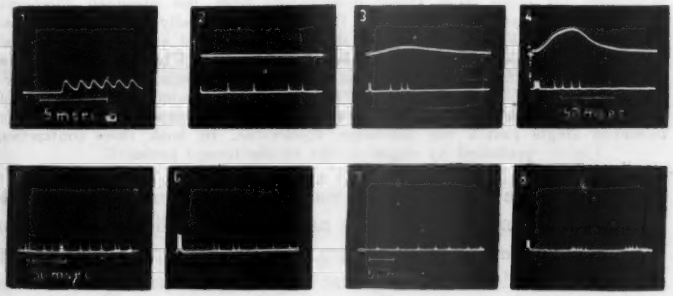

Fig. 8. Experiment suggesting convergence of excitatory action by Golgi and spindle afferents. Record 1 shows discharge in DSCT fibre on single shock stimulation of the triceps nerve. In records 2—4 the resting tension was approximately 500 g and on isometric single twitch of gastrocnemius the discharge accelerates (stimulation of triceps nerve). To obtain 5—6 the tendon was pulled strongly by hand and with contraction of the muscle the discharge now pauses. In records 7—8 a weight of 500 g was attached to the gastrocnemius tendon and after contraction of the loaded muscle an intermittent discharge appears.

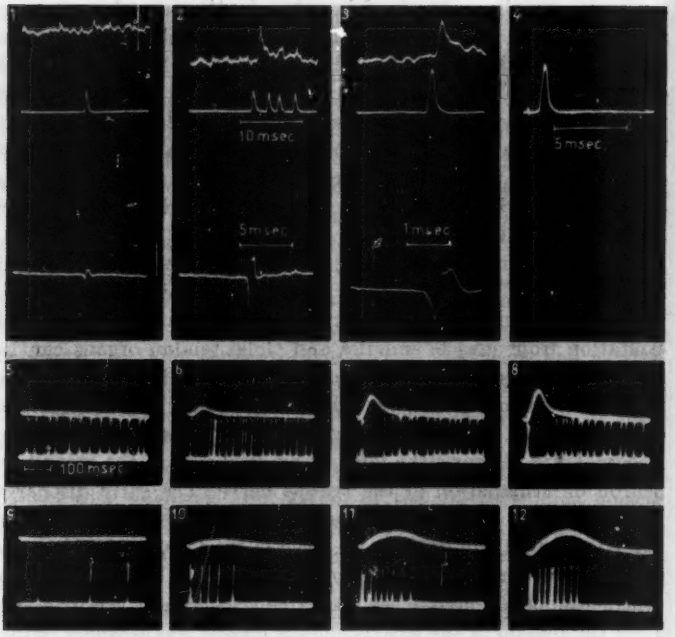

Fig. 9. Convergence of excitatory action by primary Golgi tendon organ and muscle spindle afferents. In records 1—3 stimulation of the triceps nerve and recordings (in order from above downwards) from the dissected Flechsig's fasciculus, intra-axonally from DSCT fibre and triphasically from L VII dorsal root entry zone. Faster sweep speed in record 3. Record 4 shows intra-axonal spike on antidromic stimulation of dissected fasciculus. The neuron discharges on stretch of gastrocnemius as well as of the soleus muscle. Stretch of gastrocnemius (records 5—8) with pause of discharge during isometric contraction of this muscle. To obtain records 9—12 the soleus muscle was stretched and with isometric single twitch the discharge accelerates. In both cases contraction provoked by single shocks to the triceps nerve.

steady pull the muscle nerve was stimulated in record 6. The contraction is not as isometric as when the tendon is fixed to the strain gauge and there is now a pause during the contraction of the muscle. However, the pause might not necessarily be indicative of excitatory action from muscle spindles. Other possibilities are that it might be due either to a refractory state of the secondary neuron after the burst of impulses elicited from single shock stimulation of the muscle nerve or it may be due to refractoriness in the peripheral terminals of the primary afferents. Actually it was observed in control experiments on Golgi tendon

organ afferents that a pause of this duration may follow single shock stimulation of the muscle nerve when contraction is not isometric. Records 7—8 are also from the same neuron. A string attached to the tendon of the muscle was loaded with a 500 g weight (record 7). On contraction there is a pause and after contraction an intermittent discharge of the same type as seen in Fig. 7 record 8, and described previously (LAPORTE et al. 1956 c) as being similar to the discharge in primary afferents with annulospiral endings. We have now made further control experiments with recording from single fibres in the dorsal roots and consistently found intermittent discharge of this type in primary afferents connected with muscle spindles, but not in those of Golgi tendon organs. The occurrence of the intermittent discharge in this neuron suggests that it receives excitatory action from muscle spindles as well.

Stronger indication of such convergence is found in the experiment illustrated in Fig. 9. Records 1—4 are shown for the identification of the neuron. As appears in record 1 there is excitatory action by low threshold group I afferents in the triceps nerve. This neuron could be discharged on stretching the soleus as well as the gastrocnemius muscle. On isometric contraction of the gastrocnemius muscle the discharge pauses (records 5—8), whereas it accelerates during isometric contraction of the soleus muscle (records 9—12).

Table 3.

Receptors contributing main excitatory action to individual DSCT neurons as disclosed by discharge during contraction of muscle.

Muscle	Spindle	Golgi	Spindle + Golgi
Gastrocnemius..	15	7	1
Soleus	10	10	
Quadriceps	24	11	1
	49	28	2

The results of the experiments with adequate stimulation are summarized in Table 3, which includes only the results of the later experiments, when the technique of examination had been

improved and when all neurons found were examined. It appears that almost all neurons discharged as if they received excitatory action from only one type of receptor, and that 36 per cent of these were of the Golgi type.

3. Problems concerning convergence of excitatory and inhibitory actions from one muscle.

LAPORTE et al. (1956 c) found that some DSCT neurons may discharge on electrical stimulation of group I afferents from a certain muscle and nevertheless be inhibited by stretch of the same muscle. We have confirmed this finding and now observed that this inhibitory action may appear even with a slight stretch of the muscle. Furthermore we have been able to reproduce this phenomenon using electrical stimulation and found that also the inhibitory action may be carried by group I afferents. This has been found both with single volleys and with repetitive stimulation. The records in Fig. 10 were obtained from a neuron in which a double spike appeared with a group I volley (records 1—3). It was not possible to decide whether the first spike arose with the nearly maximal Ia volley or with a weak Ib volley. The second spike clearly results from a weak Ib volley. With a somewhat larger Ib volley the second spike is inhibited to appear again after a longer latency (records 5—6) with further increase of the stimulus strength. When decreasing the primary afferent volley this sequence of inhibition and excitation occurred in the reversed order. The group II volley can be seen in the triphasic recording from the dorsal root entry zone in record 6. In record 4 there is no sign of activation of group II and consequently it is concluded that the inhibitory effect described is mediated by group I fibres. In two other cases inhibitory action by a single group I volley was found to be signalled by lengthening of the interval between the two spikes with increase of the primary afferent volley.

Similar findings were made with the use of repetitive stimulation. In two instances neurons were found which were monosynaptically excited by a group I volley from a certain muscle nerve (severed peripherally) and nevertheless their resting discharge was inhibited on repetitive stimulation at 100/sec of group I fibres in the same nerve.

LAPORTE et al. (1956 c) raised the question whether the occasional co-existence of inhibitory and excitatory action to DSCT

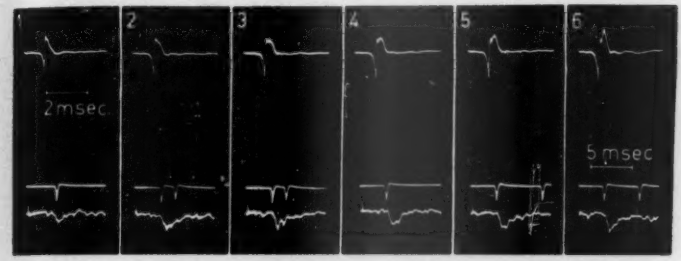

Fig. 10. Inhibition of second spike by increase of the primary afferent volley. Stimulation of the biceps p. + semitendinosus nerves and simultaneous recordings (in order from above downwards) triphasically from the L VII dorsal root entry zone, intra-axonally from DSCT fibre and from the dissected fasciculus.

neurons found was a regular feature of the synaptic organization which might account for the unexpectedly low frequency of the post-synaptic discharge so often encountered on adequate activation of the muscle stretch receptors. If this were the case the possibility should be considered that antagonistic synaptic actions originate from different types of receptors. Since it now is established that also the inhibitory action may be carried by group I afferents the question is whether neurons discharged by spindle afferents receive inhibitory action from Golgi tendon organ afferents, and conversely that neurons discharged by tendon organ afferents are inhibited from spindle afferents. That this would be a regular pattern of organization appears exceedingly unlikely in view of the two preceeding sections of this paper demonstrating convergence of excitatory action by spindle and tendon organ afferents. As appears from the following observations we have not been able to obtain evidence in favour of such a notion.

Neurons were identified which could be adequately activated by muscle spindle afferents from a certain muscle, and the discharge on stretch of the muscle was compared before and after injection of 1 ml of a solution containing one per cent cocain in the region between the muscle and the tendon. Three successful experiments were made and in none of them was there an increase of the discharge. There is another observation to show that the low frequency of the post-synaptic discharge in neurons adequately activated by muscle-spindle afferents is not due to inhibition from Golgi tendon organs. It was shown by MATTHEWS (1933) that muscle spindle afferents but not Golgi tendon organ afferents respond to a quick stretch of the muscle. The frequency

of the discharge in a great number of DSCT neurons activated by muscle spindle afferents was compared during a quick stretch of the muscle and during steady pull. The frequency of post-synaptic discharge in the individual neuron was always higher during a quick stretch but in proportion to the frequency reached during a steady pull. When a neuron discharged at high frequency during a quick stretch, it always responded well also to a steady pull of the muscle, suggesting that the low frequency so often encountered even at strong adequate activation merely is due to inefficient excitatory action by the muscle spindle afferents. Furthermore it should be noted that we never found a neuron responding to light stretch with excitation and to stronger stretch with inhibition.

Similarly there are indications that DSCT neurons discharged by Golgi tendon organ afferents, as evidenced by acceleration of discharge during isometric contraction, do not generally receive inhibitory action from muscle spindle afferents. The neurons activated by Golgi tendon organ afferents have often a resting discharge. It has never been possible to inhibit this resting discharge by a slight stretch or by a quick stretch of the muscle as might expectedly have been the case if fibres with annulo-spiral endings carried inhibitory action to these neurons.

Discussion.

Previous experiments were concerned with the synaptic connection of group I and II muscle afferents to neurons with ascending axons in the dorso-lateral funiculus (LAPORTE, LUNDBERG and OSCARSSON 1956 a, b, LAPORTE and LUNDBERG 1956). In the present work an attempt has been made to analyse the connections of the two afferent systems within group I, the muscle spindle afferents (Ia) and the Golgi tendon organ afferents (Ib).

For this analysis the subdivision of the group I spike from some thigh muscles as described by BRADLEY and ECCLES (1953) has proved itself most useful. The mass discharge in the dissected Flechsig's fasciculus does not attain its maximal size on stimulation of all the low threshold fast conducting fibres (Ia volley) but increases discontinuously when the high threshold group I fibres (Ib volley) are included in the primary afferent volley. Furthermore, when recording from single axons in the Flechsig's fasciculus, neurons were found which were activated only by a

Ib volley. Considering the evidence given by BRADLEY and ECCLES these findings are strongly indicative of excitatory action by Golgi tendon organ afferents as well as by muscle spindle afferents as was subsequently proved in experiments with adequate stimulation of stretch receptors.

LAPORTE and LUNDBERG (1956) in similar experiments gave evidence for excitatory action by muscle spindle afferents to some neurons and in the present experiments 36 per cent of the neurons turned out to be activated by Golgi afferents as evidenced by acceleration of the discharge during isometric contraction of the muscle. It is of interest that HUNT (1954) found that of the group I afferents from soleus 44 per cent were connected to Golgi tendon organs against 28 per cent from the medial gastrocnemius. Among the DSCT neurons discharged on stretch of soleus a higher percentage receive excitatory action from Golgi tendon organ afferents than was found for the neurons discharged from gastrocnemius or quadriceps.

LAPORTE et al. (1956 b) defined the DSCT as the axons in the dorso-lateral funiculus of neurons which receive excitation by mono-synaptic action of group I muscle afferents. According to the present results the DSCT has two subgroups. There is good reason to assume that both these subgroups have their cells in Clarke's column. The Ia and Ib primary afferent volleys can be recorded from the dorsal column in the region of the lower end of Clarke's column as found by LLOYD and MCINTYRE (1950) for the group I volley. It is not likely that a closer comparison of the decrease in conduction velocity will give more detailed information on the site of relay because the ventral spino-cerebellar tract, relaying at a lower level, is supplied by afferents from Golgi tendon organs but not from muscle spindles (OSCARSSON 1956).

In the experiments with electrical stimulation of the muscle nerve it was found that a great number of the neurons examined were activated both by the low and high threshold fibres within group I. The question as to whether this convergence may be taken as evidence for convergence of excitatory action from Golgi tendon organs and muscle spindles must be judged with considerable caution.

For establishment of the functional equivalents of these two fibre groups, BRADLEY and ECCLES (1953) used the mono-synaptic connections to and direct inhibition of motoneurons as index.

The low threshold fibres turned out to have the segmental connections expected from spindle afferents. However, the situation might be different in a system in which little summation would be necessary for excitation and less summation is needed in Clarke's column cells than in the motoneurons. Some convergence could be accounted for by the occasional occurrence of afferents from Golgi tendon organs in the low threshold component of group I and vice versa of fibres with annulo-spiral endings within the high threshold component. The fact that also group II fibres carry excitatory action to DSCT neurons contributes additional uncertainty because even with absence of group II components in the dorsal root filament electrogram it cannot be excluded that some group II fibres were stimulated with Ib. Furthermore it should be considered that a group I volley sometimes gives a double spike response. This indicates that excitatory action survives the first spike and may in the experiments with double volleys aid the excitatory action by the later incoming test volley. If convergence of excitatory action by Ia and Ib volleys as examined in the present investigation had only been encountered occasionally we would not have attached much significance to the finding, but the fact that it was found so often makes it in our opinion likely that Golgi tendon organs and muscle spindle afferents may supply excitatory action to the same Clarke's column cell and evidence for such convergence was also found in the experiments with adequate stimulation. However, too many factors of uncertainty are involved in the interpretation of the results obtained on recording from fibres to allow conclusion about the degree of convergence.

The existence of convergence of excitatory action from Golgi tendon organ and muscle spindle afferents must not necessarily be of any great significance with regard to the forwarding of messages to the cerebellum. It was reported by LAPORTE and LUNDBERG (1956) that neurons excited by afferents from a certain muscle sometimes failed to respond on adequate activation of stretch receptors. A neuron must receive a larger number of afferents to discharge on adequate receptor activation than is needed for activation by a synchronous volley (cf. HOLMQVIST, LUNDBERG and OSCARSSON 1956). Excitatory actions disclosed as post-synaptic discharge on electrical stimulation may therefore very well be subsidiary and of little significance with adequate activation. Actually the present experiments with adequate stim-

ulation indicate that the great majority of neurons subserve transmission of information only from one receptor system, either Golgi tendon organs or muscle spindles. The possibility should be mentioned that among the latter neurons there may be two groups, one with predominant activation from group I fibres (nuclear bag-endings) and the other from group II fibres (myotube endings). However, HUNT (1954) has shown that the adequate stimulus for these two endings does not differ qualitatively. There is some quantitative difference, the threshold for adequate activation of group II fibres being higher, but this difference is not great enough to suggest that with two lines from muscle spindles to the cerebellum the range of sensitivity would be widened significantly.

Co-existence of inhibitory and excitatory actions from one muscle to some DSCT neurons was observed by LAPORTE and LUNDBERG (1956). It has now been found that both these actions may be mediated by group I afferents. It may, however, be doubted if this convergence is representative of the general pattern of organization. It does not seem likely that convergence of excitatory and inhibitory actions mediated by afferents connected to the same type of receptors occurs commonly. Even though the results presented in this paper indicate that Ia and Ib afferents may carry excitatory action to the same neuron, the possibility should be envisaged that on analogy with the motoneurons (GRANIT 1950, LAPORTE and LLOYD 1952, HUNT 1952), the reciprocal effects from one muscle are carried by afferents from different receptors. However, the present experiments have given no evidence for the general occurrence of opposite actions by afferents from muscle spindles and Golgi tendon organs; nor is there any reason why the Clarke's cells and the motoneurons should be analogous in this respect.

It is therefore suggested that this convergence of inhibitory and excitatory actions from one muscle is exceptional. It will be demonstrated in the next paper (HOLMQVIST, LUNDBERG and OSCARSSON 1956) that DSCT neurons receive inhibitory action mediated by group I afferents from an extensive peripheral field including other muscles than the antagonists. Presumably convergence of excitation and inhibition shows up when a neuron receives subsidiary excitation from a certain muscle. Perhaps the inhibitory action in such cases subserves spatial discrimina-

tion by limiting strey excitation from muscles other than the one that is the main source of afferents.

Summary.

Intra-axonal recording in Flechsig's fasciculus was used to study neurons which could be activated by electrical stimulation of group I muscle afferents. There were recorded simultaneously the mass discharge in the dissected Flechsig's fasciculus and sensory volleys with triphasic recording from dorsal roots for control of input in terms of Ia and Ib volleys. In addition adequate stimulation of stretch receptors in muscles was used in some experiments.

Ib fibres reach the region of Clarke's column via the dorsal column as do the Ia fibres.

Most of the mass discharge in the dissected Flechsig's fasciculus appears with a primary Ia volley but some increase occurs with Ib. Most neurons examined discharge on stimulation of Ia, but some only when stimulation was raised to include part of Ib. In many neurons excited by Ia, a second post-synaptic spike appeared with a Ib volley generated in the refractory period of the preceding Ia volley. Ia as well as Ib volleys were found to contribute inhibitory action to neurons excited by group I afferents from antagonist muscles.

The discharge in secondary neurons, responding to a stretch of a muscle, was examined during contraction of the muscle (soleus, gastrocnemius or quadriceps). An acceleration was found in 36 per cent, demonstrating excitatory action by Golgi tendon organ afferents, and the remainder paused during contraction. Occasionally results were obtained indicating convergence of excitatory action from Golgi tendon organ and muscle spindle afferents.

It is concluded that some DSCT neurons subserve transmission of information from Golgi tendon organs and others from muscle spindles. The occurrence of convergence of excitatory action from muscle spindles and Golgi tendon organs is discussed.

Convergence of excitatory and inhibitory actions mediated by group I afferents from the same muscle has been encountered in some cases. It is suggested that convergence of this type is exceptional and not representative for the general pattern of synaptic organization.

Acknowledgement: The expenses of this work were defrayed by grants from Stiftelsen Therese och Johan Anderssons Minne and from the Medical Research Council. Technical assistance was given by Miss RUTH ARALDSSON.

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Institute of Physiology, University of Lund, Sweden.

Functional Organization of the Dorsal Spino-Cerebellar Tract in the Cat.

V. Further Experiments on Convergence of Excitatory and Inhibitory Actions.

By

B. HOLMQVIST, A. LUNDBERG and O. OSCARSSON.

Received 2 August 1956.

Previous papers in this series have been concerned with identification of primary afferents making monosynaptic connections with dorsal spino-cerebellar tract (DSCT) neurons and also with inhibitory action converging onto these neurons (LAPORTE, LUNDBERG and OSCARSSON 1956 a, b, LAPORTE and LUNDBERG 1956, LUNDBERG and OSCARSSON 1956).

In the present paper will be presented results showing that DSCT neurons receive excitatory action from interneurons, which presumably is largely responsible for the resting discharge often found in these neurons. In addition convergence of inhibitory and excitatory action from different nerves has been examined in greater detail than previously. It will be shown that the receptive field supplying monosynaptic excitatory action usually is rather limited. Convergence of inhibitory action, however, is much more extensive and not limited to the antagonist muscle of the one supplying excitation.

Results.

1. Excitatory action from interneurons.

It was found by LAPORTE and LUNDBERG (1956 c) that in many of the neurons examined there was a resting discharge usually with a frequency of about 10/sec. With intact afferent innervation this discharge might be due to impulses from recep-

tors. However, the resting discharge was found in DSCT neurons after denervation of the leg. Since the hip muscles were not denervated, the possibility remained that the resting discharge was caused by afferent inflow from these muscles converging to the neurons examined (which could be excited by electrical stimulation either from the quadriceps or the biceps p. + semitendinosus nerve). Experiments reported in the present paper show that convergence of excitatory action to DSCT neurons is not very extensive, and it is therefore likely that the other alternative pointed out by LAPORTE and LUNDBERG is the more likely one, *i. e.* the existence of an excitatory bombardment from interneurons. In order to settle this question experiments were performed after ipsilateral section of dorsal roots caudally of Th XIII. For the actual experiments dorsal roots were stimulated with the usual recording from the dissected Flechsig's fasciculus and intraaxonally from fibres of the tract. Only those neurons were considered in which a post-synaptic spike appeared with stimulation of low threshold afferents. In most of these neurons a second spike appeared at the usual interval at a stimulus strength slightly supramaximal to that giving a maximal synchronous volley in the dissected fasciculus. Responses with these characteristics have been obtained on stimulation of muscle nerves, but not of other peripheral nerves.

In records 1—4, Fig. 1, the dorsal root L VII was stimulated with increasing strength and the response accords with the specifications mentioned above. On antidromic stimulation of the dissected fasciculus the intra-axonal electrode recorded a spike after 0.6 msec and thus the first of the orthodromically elicited spikes corresponds to the front of the mass discharge recorded in the dissected fasciculus. Record 5 shows the resting discharge in this neuron with a frequency higher than 10/sec. Repetitive stimulation of the L V dorsal root was started during the sweep shown in record 6 and interrupted about 2 sec later when record 7 was taken. During stimulation the discharge was inhibited and afterwards there was a rebound. In record 8 obtained 2 sec afterwards the resting frequency has reappeared. Incidentally the inhibition shows that the discharge was not caused by an injury to the axon at the site of recording.

Three experiments were made and in all of them neurons were found discharging at rest. In one experiment in which 17 neurons were encountered a resting discharge was found in 11. The mean

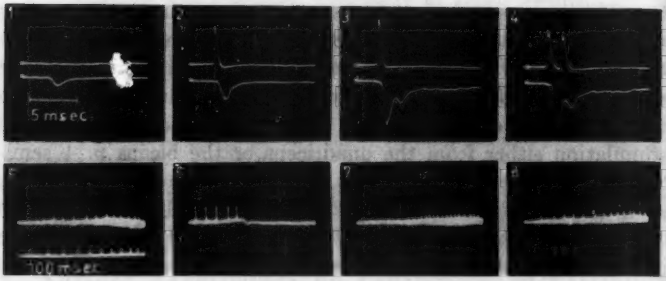

Fig. 1. Persistence of resting discharge in DSCT neuron after ipsilateral section of lumbar and sacral dorsal roots. In records 1—4 stimulation with increasing strength of L VII dorsal root and recording intra-axonally from DSCT fibre (upper trace) and from the dissected Flechsig's fasciculus. Record 5 shows the resting discharge in absence of stimulation. Inhibition of discharge with repetitive stimulation of L V dorsal root started in record 6 and interrupted in record 7 two seconds later.

frequency for all neurons was 3/sec. For comparison it may be mentioned that, in one preparation with intact dorsal roots but denervated leg, 10 neurons were found which could be monosynaptically excited by group I afferents either from the quadriceps or from the biceps p. + semitendinosus nerve and all of these neurons had a resting discharge, the mean frequency being 14/sec. In another similar experiment 8 out of 10 neurons discharged in the absence of any electrical stimulation, the mean frequency for all neurons being 8/sec. It is noteworthy that in many of the silent neurons a 30 sec period of stimulation at 100/sec of the dorsal root resulted in activity lasting for several minutes after the end of the stimulation. It is concluded that the discharge is caused by interneurons and is not merely spontaneous activity in the DSCT neurons.

Dorsal root section was ipsilateral, but it can be excluded that the resting discharge remaining is due to an inflow from contralateral fibres with monosynaptic connections to these neurons because, in a great number of control experiments with stimulation of the contralateral sciatic nerve, monosynaptic excitation was never found, which is in agreement with what could be expected from the findings by SZENTAGOTHAI and ALBERT (1955).

There may be several reasons for the drop in frequency after dorsal root section. Probably withdrawal of monosynaptically impinging impulses is of minor importance. Withdrawal of impulses feeding the internuncial circuits responsible for the excita-

tory action may be important, but another factor may very well be interference with the blood supply of the spinal cord. Even during the course of an experiment with intact dorsal roots the resting discharge was found to have low frequency or to be absent in neurons that were encountered late in the experiment.

It was also attempted to examine the effect of myanesin on the resting discharge in DSCT neurons. Usually the changes in circulation ensuing on injection of this drug caused loss of the unit, but in one case the fibre was kept under observation and 25 mg/kg myanesin intravenously decreased the resting discharge from 8 to 3/sec.

2. Convergence of excitatory and inhibitory actions from various nerves.

The effect of afferent inflow from different sources has been examined with the use of electrical stimulation of a number of peripheral nerves as well as with adequate activation of stretch receptors in a number of muscles.

In the latter experiments neurons were searched for, which could be activated on stimulation of a given muscle nerve and discharged on stretch of the same muscle. The effect on this neuron of stretching other muscles was thereafter examined. One regular outcome of these experiments was the inhibition from antagonist muscles (cf. LAPORTE et al. 1956 b, c; LUNDBERG and OSCARSSON 1956). The effects from other muscles were not consistent. Inhibition was often encountered from other muscles than the antagonists. Sometimes a neuron could be discharged from several muscles, but in almost all experiments one muscle could be distinguished which on stretch gave a more marked increase of discharge in the neuron.

The influence of adequate activation of stretch receptors in the gastrocnemius-soleus muscles was examined on 13 neurons which were excited from quadriceps on electrical stimulation of group I afferents as well as by adequate stimulation of stretch receptors in this latter muscle. Of these neurons 7 were not influenced, 5 were inhibited and only 1 was discharged on stretch of gastrocnemius-soleus. Of 20 neurons excited on electrical stimulation of group I afferents in the triceps nerve and discharging on stretching the gastrocnemius-soleus muscles 6 were uninfluenced, 10 inhibited and 4 discharged on stretching the quadriceps muscle.

Among neurons monosynaptically activated from the triceps nerve, some discharged on stretching both the gastrocnemius and the soleus muscles; some could only be discharged from one of the muscles and were not affected by stretch of the other muscle, but some neurons discharged by stretching one of the muscles and were inhibited from the other muscle.

Table 1 summarizes the results of two experiments (I and II) with single shock stimulation of the severed muscle nerves listed and recorded from single axons as usually. In the table + denotes monosynaptic excitation by group I afferents and (+) excitation from single shock stimulation with uncertainty either of monosynaptic transmission or group I origin of the excitatory action.

Convergence of excitatory action from quadriceps and sartorius was common in experiment I but with this exception convergence was only found to 15 neurons out of 40. In some cases there was monosynaptic excitation from three different muscle nerves. Quite clearly monosynaptic connection to a certain DSCT neuron can be made by afferents from non-synergist muscles.

The records from Fig. 2 are from a neuron which could be fired by single shock stimulation both from the triceps nerve (records 1—2) and from the quadriceps nerve (records 5—6). Records 3—4 show the discharge with repetitive stimulation of the triceps nerve. In record 4 the primary afferents are stimulated at a rate of 460/sec and the secondary neuron follows this frequency. Usually DSCT neurons follow 500/sec for several seconds, but not 600/sec except initially. On repetitive stimulation of the quadriceps nerve at about 200/sec the post-synaptic neuron does not follow this frequency for more than three impulses (record 7). Presumably the neuron receives a smaller number of terminals from this nerve. Record 8 finally was obtained from the same neuron when stimulating the nerve of the plantaris muscle. Single shock stimulation of this nerve did not elicit a post-synaptic discharge but with the second impulse a post-synaptic spike appears. To obtain record 8 stimulation was supramaximal for group I fibres but the same excitatory effects were achieved when using a stimulus strength activating less than half of group I. On a few occasions neurons have been found which responded equally well from two muscles.

On the whole convergence of inhibitory action to a certain neuron is much more often encountered than convergence of excitation. This is illustrated in Table 2 which lists all the neurons

Table 1.

Convergence of excitatory action from different muscles.

	Nr.	rest. freq.	quadr.	sart.[1]	biceps ant.[2]	biceps p. + semit.	semi-membr.	triceps	plant.	flex. dig.l.[2]
	1	0	+	(+)						
	2	0	+							
	3	10	+							
	4	6				+				
	5	5	+	(+)						
	6	5	+							
	7	2	+	(+)						
I	8	5	+	(+)						
	9	0	+	(+)						
	10	6				+				
	11	12	+	+						
	12	0				(+)				
	13	6	(+)	(+)						
	14	0	+	(+)						
	15	2	+	(+)						
	16	0	(+)	+						
	17	7	+							
	18	2	+							
	19	0	+							
	20	4	+					+		
	21	0			(+)		+			
	22	1	+							
	23	2				+				
	24	0							+	
	25	10								+
	26	0	+					+		
	27	1				+				
	28	0			+	+		(+)		
	29	0	+					+		
II	30	5	+							
	31	0	+					+		
	32	0	+							
	33	4	+		+			+		
	34	0	(+)					+		
	35	0						+	+	
	36	0	+							
	37	0				+				
	38	5						+		
	39	0				+		+	+	
	40	2	+							

[1] The sartorius nerve not dissected in experiment II.

[2] The anterior biceps and flexor digit. long. nerves not dissected in experiment I.

fired by single shock stimulation of the quadriceps or the biceps p. + semitendinosus nerves encountered in two experiments (I and II) which had a resting discharge and thus could be easily tested for convergence of inhibitory action. In the table s means

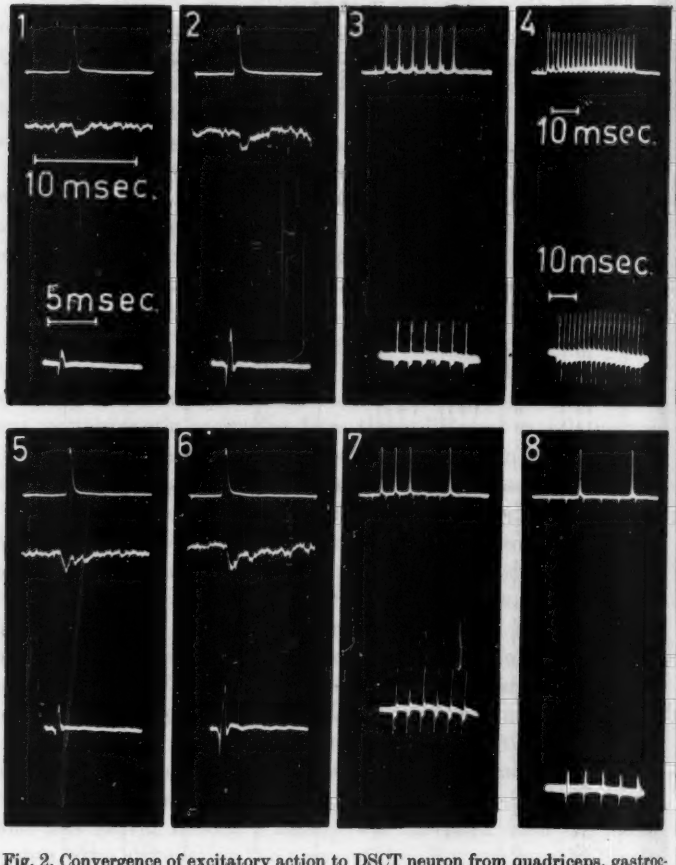

Fig. 2. Convergence of excitatory action to DSCT neuron from quadriceps, gastrocnemius-soleus and plantaris nerves. In record 1 and 2 single shock stimulation of the triceps nerve and in records 5—6 of the quadriceps nerve. With simultaneous recording in order from above downwards from DSCT fibre, from the dissected Flechsig's fasciculus and triphasically from the L VII dorsal root entry zone. Record 3 and 4 were obtained with repetitive stimulation of the triceps nerve, 7 of the quadriceps nerve and 8 of the plantaris nerve.

monosynaptic excitation from single shock stimulation and (s) excitation from single shock stimulation with uncertainty either about monosynaptic transmission or group I origin of excitation. Repetitive stimulation at 100/sec was used for testing convergence and — denotes decrease or cessation of discharge and + acceleration. The stimulus strength was adjusted to give a volley of about

two thirds maximal group I as recorded triphasically in the dorsal root entry zone. All the effect must be due largely or exclusively to activation of group I fibres. Neurons excited by single shocks accelerated on repetitive stimulation when no mention of inhibition is given in the table.

It appears quite clearly from the table that inhibitory action can be elicited from other nerves than the nerve of the muscle antagonistic to the one supplying monosynaptic group I excitation. Of 11 quadriceps neurons 6 were more or less inhibited from the semimembranosus, triceps and plantaris nerves, against all from the antagonist biceps p. + semitendinosus nerve.

Neurons 7 and 16 discharged on single shock stimulation of group I afferents in the quadriceps nerve, although with such a long latency that transmission may have been polysynaptic. Repetitive stimulation of group I fibres in the quadriceps nerve in this case gave inhibition. Similar findings have been made on neurons in which the brief latency of the discharge proved transmission to be monosynaptic (cf. LUNDBERG and OSCARSSON 1956).

Neuron 8 in Table 2 accelerated on repetitive stimulation of the semimembranosus nerve although it could not be fired on single shock stimulation of this muscle. To account for the observed facilitation in this case one could assume that this neuron received no or little inhibitory action from this nerve so that a small excitatory action would be effective. In experiments with adequate stimulation it has similarly been noted that a neuron which could not be fired by strong single shock stimulation of a certain muscle nerve nevertheless sometimes may exhibit a small discharge on stretch of the same muscle. Temporal summation of monosynaptic excitatory action may account for the acceleration, but another possibility complicating the interpretation of these experiments is that the interneurons referred to in part 1 of this paper may be activated by muscle afferents.

It may be asked whether there is some feature distinguishing inhibitory action by antagonist muscle nerves from that elicited by another nerve. On cessation of repetitive stimulation of the nerve from an antagonist nerve there is rapid resumption of the discharge and very often rebound. This happens quite frequently also after inhibition provoked from a non-antagonistic muscle nerve, but in the latter case inhibition has on some occasions been found to outlast stimulation for several seconds, gradually

Table

Convergence of inhibitory

	Nr.	rest. freq.	quadr.	sart.[1]	biceps[2] ant.	biceps p. + semit.
I	1	10	s	–		– – –
	2	5	s	(s)		– – –
	3	5	s	– – –		– – –
	4	2	s	(s)		– – –
	5	5	s	(s)		– – –
	6	12	s	s		– – –
	7	2	(s) – – –	(s) – –		– – –
II	8	7	s		+	– – –
	9	17	s		o	– – –
	10	5	s		–	– – –
	11	2	s		– – –	– – –
I	12	6	– – –	– –		s
	13	6	– – –	– –		s
II	14	1	– – –		o	s
	15	1	– – –		– – –	s
I	16	7	(s) –	(s) – –		s

[1] The sartorius nerve not dissected in experiment II. — [2] The anterior biceps

resuming the resting frequency. Similarly it has been found that on single shock stimulation inhibition may sometimes last as long as 100 msec and appear as a distinct pause when the frequency of the resting discharge is high. However, these findings are rather exceptional. Usually there has been no evidence for discrimination of inhibition from antagonist and non-antagonist nerves.

It appears also from Table 2 that the resting discharge sometimes can be influenced on repetitive stimulation of cutaneous nerves. This finding is not unexpected considering the fact that the DSCT neurons receive excitatory action from interneurons.

3. Adequate activation of neurons receiving excitatory action from skin and from high threshold muscle afferents.

LAPORTE et al. (1956 b) reported that some neurons with axons in Flechsig's fasciculus could only be discharged from muscle nerves if group II and III fibres were stimulated. Characteristically there was often convergence from skin and from other muscles (including antagonists). We have now examined the discharge in these neurons with adequate

2.

action from different muscles.

semi-membr.	triceps	plant.	flex. dig. l.[2]	saph.	per. sup.
–	– – –	– –		o	–
– – –	– – –	– –		+ +	o
– –	– – –	– – –		o	o
– – –	– – –	– – –		– – –	+ +
–	– – –	– – –		o	+ +
o	o	–		o	o
–	o	o		+ +	– – –
+ +	– – –	– – –	o	+ +	+ +
+	– –	o	o	o	o
–	– – –	–	o	o	o
–	o	o	+	o	+
o	– – –	– –		o	o
– –	o	o		o	o
– – –	o	o	+	o	+
– – –	– – –	o	o	o	o
s	s	(s)		+ +	– –

and flexor digit. long. nerves not dissected in experiment I.

activation of muscle stretch receptors. Identification of these neurons was established by the occurrence of a longlasting repetitive discharge from a stimulus which was strong enough to excite group III fibres.

The lower record in Fig. 3 shows the discharge in such a neuron on stretch of quadriceps. The resting discharge was about one per sec and a load of 500 g was attached to the quadriceps tendon during the time indicated by the white line. After release of the pull the frequency of the discharge decreased only slowly so that 17 sec later it was still about three times the resting frequency. For comparison there is shown in the upper record in Fig. 3 the discharge on stretch in a neuron which received excitatory action from group I afferents. On release of the stretch there is a pause and slow resumption of the resting frequency.

The characteristic pattern of discharge in the neurons activated by high threshold muscle afferents makes it possible to distinguish them solely by the use of adequate stimulation. We have now further examined convergence on to them from different muscles. Convergence was indeed found to be extensive, as illustrated by the finding that in one experiment all six neurons encountered were made to discharge on stretch of the following muscles: quadriceps, biceps, semitendinosus, gastrocnemius, soleus, plantaris, flexor digitorum longus, extensor digitorum longus and peroneus longus. On electrical stimulation of

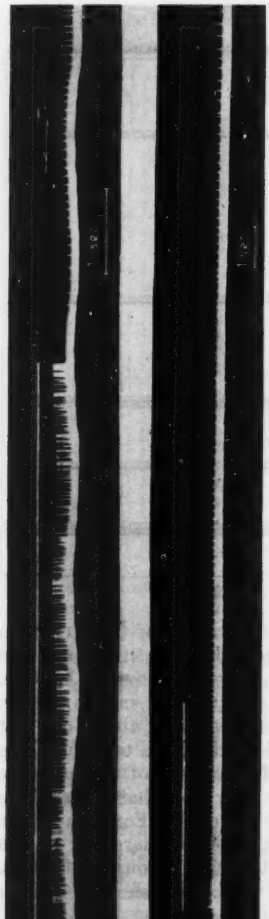

Fig. 3. Discharge with adequate activation of muscle stretch receptors in DSCT neuron (upper record) and in neuron receiving excitatory action only from high threshold muscle afferents (lower record). The DSCT neuron responded to a stretch of the extensor longus digitorum muscle, the other neuron on stretch of the quadriceps muscle. Duration of stretch shown by white line.

the quadricep nerve the typical longlasting discharge resulted with activation of high threshold fibres. Furthermore, all these neurons were also activated on stimulation of the saphenous nerve and the superficial peroneal nerve at the ankle. In no case was a neuron of this type found to be activated from only one muscle, and although some times excitation from a few muscles dominated, it is difficult to see how this system of neurons could transmit information concerning localisation. We have no knowledge concerning the end stations of these axons, but one possibility to consider is the spino-olivary tract (cf. GRUNDFEST and CARTER, 1954, DIBIAGIO and GRUNDFEST, 1955).

LAPORTE et al. (1956 b) encountered neurons with axons in Flechsig's fasciculus, which could only be activated by skin afferents. Since in their experiments only two muscle nerves were used, these neurons could not with certainty be distinguished from those which received excitatory action from both skin and muscle nerves.

In view of the findings reported in this section it is now clear that they form a group of their own (cf. MORIN and GARDNER, 1955; MORIN, 1955).

Discussion.

The present investigation has made it clear that DSCT neurons receive excitatory action from interneurons, which presumably maintains the resting discharge so fre-

quently found in these cells. The functional significance of this activity may be twofold. The excitability of the cells is maintained at a level permitting the discharge in primary afferents with mono-synaptic connections to be transmitted on adequate activation. Furthermore the resting discharge may be requisite as a background for forwarding information from antagonists and other muscles, which may work through the inhibitory actions disclosed. That fibres from interneurons terminate on Clarke column cells is known from the work of SZENTAGOTHAI and ALBERT (1955). That some of these are excitatory is clear from the present work, but this conclusion does not change the supposition that some may be inhibitory as was suggested by LAPORTE et al. (1956 b) on analogy with the findings on motoneurons by ECCLES, FATT and LANDGREN (1954, 1956).

Convergence of excitatory action by group I afferents from different muscles was described by LAPORTE et al. (1956 b), and it is now clear that, although with excitation as a criterion often no convergence is found, some neurons may be excited monosynaptically from as many as three different muscles. On repetitive stimulation as well as with adequate stimulation of stretch receptors a neuron usually responded well only from one muscle, which thus would represent the centre of the receptive field for that particular neuron. It is of interest that to some DSCT neurons monosynaptic connections are made by group I afferents from muscles, which not are synergists as for example quadriceps and triceps respectively biceps p. + semitendinosus and triceps. It should also be recalled that occasionally neurons have been found to receive excitatory action from antagonist muscles (LAPORTE et al. 1956 b, LUNDBERG and OSCARSSON 1956).

On the whole convergence of inhibitory action to DSCT neurons is much more impressive than convergence of excitatory action. Inhibitory actions from antagonist muscles have been considered in earlier papers, and the present work has shown that in addition a given neuron receives inhibitory action mediated by group I afferents also from other muscles than the antagonists. Inhibition from antagonist muscle is usually more marked but it is not certain that it should be distinguished from the inhibitory action supplied by non-antagonist muscles as is possible in motoneurons (GRANIT 1950, LAPORTE and LLOYD 1952).

Throughout the work on the dorsal spino-cerebellar tract axonal

recording has been used. This technique of recording is undoubtedly favourable for work on functional organization since the cell bodies are not interfered with and many neurons may be investigated, but has the disadvantage of offering only indirect information about synaptic processes. A noticeable feature in transmission to Clarke's column is that with repetitive stimulation impulses may be transmitted at a very high frequency. In this respect Clarke's column is similar to the cuneate nucleus (THERMAN 1941, AMMASSIAN 1956) and also to the intermediate nucleus (ECCLES, FATT and LANDGREN 1956), but not to the lateral geniculate body (BISHOP 1953, BISHOP and MCLEOD 1954).

From experiments involving stimulation of dorsal roots LLOYD and MCINTYRE (1950) concluded that much less summation was needed for excitation of Clarke's cells than for motoneurons. Further support for his conclusion has now been obtained in experiments with single fibre recording when a post-synaptic spike appeared with a very small afferent volley. MCINTYRE (1953) has further claimed that the Clarke's column relay "approaches a one to one transmission" and has "virtually no subliminal fringe as shown by the absence of post-tetanic potentiation". It is true that post-tetanic potentiation is small in the DSCT but we cannot agree with the statement that it is absent. We have on several occasions observed that neurons receiving subliminal excitation may be brought to discharge post-tetanically, and after tetanic stimulation the mass discharge recorded in the dissected fasciculus was found to increase by about 25 per cent. However, it is obvious that, with this small degree of post-tetanic potentiation, excitability changes in neurons of the subliminal fringe or close to the subliminal fringe cannot be used to determine the timecourse of excitatory and inhibitory post-synaptic potentials.

The fact that synaptic transmission of the adequately elicited discharge in primary afferents so often is poor does, however, suggest, not only that some summation is needed for excitation by a synchronous volley, but also that each neuron receives a rather limited number of primary afferents, and further that the excitatory post-synaptic potential is shortlasting. However, to obtain detailed informations in these matters it is necessary to record intra-cellularly from Clarke's column cells.

Summary.

Intra-axonal recording in Flechsig's fasciculus was used for a further investigation of the functional organization of the dorsal spino-cerebellar tract.

After ipsilateral section of lumbar and sacral dorsal roots, many neurons identified as belonging to the dorsal spino-cerebellar tract (neurons activated by group I muscle afferents) were found to have a resting discharge. In silent neurons a discharge could be made to appear after repetitive stimulation of dorsal roots. It is concluded that these neurons receive excitatory action from interneurons.

Convergence of excitation and inhibition onto DSCT neurons from various muscles was studied with the use of electrical stimulation of muscle nerves as well as with adequate activation of stretch receptors.

As evidenced by monosynaptic excitation on single shock stimulation of muscle nerves, convergence of excitatory action mediated by group I afferents was found to be rather limited. A number of neurons can only be fired from one muscle nerve but some from as many as three out of seven nerves (including non-synergists).

Convergence of inhibitory action mediated by group I afferents is extensive also from other muscles than the antagonist of the nerve supplying the main excitatory action.

Acknowledgement: The expenses of this work were defrayed by grants from Stiftelsen Therese och Johan Anderssons Minne and from the Medical Research Council. Technical assistance was given by Miss Ruth Araldsson.

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INDEX

Pag.

E. Asmussen and M. Nielsen, Physiological Dead Space and Alveolar Gas Pressures at Rest and during Muscular Exercise 1

B. Andersson and S. Larsson, Water and Food Intake and the Inhibitory Effect of Amphetamine on Drinking and Eating before and after "Prefrontal Lobotomy" in Dogs 22

Sv. E. Budolfsen, The Influence of Different Ions on the Intestinal Absorption of Sodium and Chloride 31

B. Folkow and B. Löfving, The Distensibility of the Systemic Resistance Blood Vessels 37

A. Lundberg and O. Oscarsson, Functional Organization of the Dorsal Spino-Cerebellar Tract in the Cat. IV. Synaptic Connections of Afferents from Golgi Tendon Organs and Muscle Spindles 53

B. Holmqvist, A. Lundberg and O. Oscarsson, V. Further Experiments on Convergence of Excitatory and Inhibitor yActions 76

Kungl. Boktr. Bibliot. 1956
563562

Pag.
1
29
31
37
53
76